US009301510B2

(12) **United States Patent**
McWhirter et al.

(10) **Patent No.: US 9,301,510 B2**
(45) **Date of Patent: Apr. 5, 2016**

(54) **MICE THAT PRODUCE ANTIGEN-BINDING PROTEINS WITH PH-DEPENDENT BINDING CHARACTERISTICS**

(71) Applicant: **Regeneron Pharmaceuticals, Inc.**, Tarrytown, NY (US)

(72) Inventors: **John McWhirter**, Tarrytown, NY (US); **Lynn MacDonald**, White Plains, NY (US); **Andrew J. Murphy**, Croton-on-Hudson, NY (US)

(73) Assignee: **Regeneron Pharmaceuticals, Inc.**, Tarrytown, NY (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/832,309**

(22) Filed: **Mar. 15, 2013**

(65) **Prior Publication Data**

US 2013/0247235 A1 Sep. 19, 2013

Related U.S. Application Data

(60) Provisional application No. 61/611,950, filed on Mar. 16, 2012, provisional application No. 61/613,352, filed on Mar. 20, 2012, provisional application No. 61/736,930, filed on Dec. 13, 2012.

(51) **Int. Cl.**

A01K 67/027	(2006.01)
C07K 16/00	(2006.01)
C12N 15/85	(2006.01)

(52) **U.S. Cl.**

CPC ***A01K 67/0275*** (2013.01); ***A01K 67/0278*** (2013.01); ***C07K 16/00*** (2013.01); ***C12N 15/8509*** (2013.01); *A01K 2217/072* (2013.01); *A01K 2217/075* (2013.01); *A01K 2217/15* (2013.01); *A01K 2227/105* (2013.01); *A01K 2267/01* (2013.01); *C07K 2317/21* (2013.01); *C07K 2317/565* (2013.01); *C12N 2800/204* (2013.01)

(58) **Field of Classification Search**

CPC A01K 2267/01; A01K 67/0275

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,545,806	A	8/1996	Lonberg et al.	
5,545,807	A	8/1996	Surani et al.	
5,603,931	A	2/1997	Raso	
5,667,988	A	9/1997	Barbas et al.	
5,999,908	A	12/1999	Abelow	
6,096,551	A	8/2000	Barbas et al.	
6,162,963	A	12/2000	Kucherlapati et al.	
6,586,251	B2	7/2003	Economides et al.	
6,596,541	B2	7/2003	Murphy et al.	
6,673,986	B1	1/2004	Kucherlapati et al.	
6,774,279	B2	8/2004	Dymecki	
6,946,548	B2	9/2005	Sarkar et al.	
7,052,873	B2	5/2006	Tsuchiya	
7,067,284	B1	6/2006	Barbas et al.	
7,105,348	B2	9/2006	Murphy et al.	
7,183,076	B2	2/2007	Arathoon et al.	
7,262,028	B2	8/2007	Van Berkel et al.	
7,276,585	B2	10/2007	Lazar et al.	
7,294,754	B2	11/2007	Poueymirou et al.	
7,435,871	B2	10/2008	Green et al.	
7,501,552	B2 *	3/2009	Lonberg et al.	800/6
7,576,259	B2	8/2009	Poueymirou et al.	
7,659,442	B2	2/2010	Poueymirou et al.	
7,910,798	B2	3/2011	Tanamachi et al.	
8,062,640	B2	11/2011	Sleeman et al.	
8,080,243	B2	12/2011	Liang et al.	
8,158,419	B2	4/2012	Lonberg et al.	
8,502,018	B2	8/2013	Murphy et al.	
2002/0088016	A1	7/2002	Bruggemann	
2003/0078385	A1	4/2003	Arathoon et al.	
2003/0108925	A1	6/2003	Dix et al.	
2004/0018626	A1	1/2004	Murphy et al.	
2006/0015949	A1	1/2006	Lonberg et al.	
2006/0015957	A1	1/2006	Lonberg et al.	
2006/0099207	A1	5/2006	Wu et al.	
2006/0141456	A1	6/2006	Edwards et al.	
2006/0199204	A1	9/2006	Dix et al.	
2007/0061900	A1 *	3/2007	Murphy et al.	800/6

(Continued)

FOREIGN PATENT DOCUMENTS

CN	1277632 A	12/2000
CN	1484 707 A	3/2004

(Continued)

OTHER PUBLICATIONS

Corbett et al. (J. Mol. Biol. 1997; 270: 587-597).*

Ippolito et al. (Journal of Experimental Medicine. Jun. 12, 2006; 203(6): 1567-1578).*

Arnold, L. et al., "Development of B-1 cells: Segregation of Phosphatidyl Choline-specific B Cells to the B-1 Population Occurs after Immunoglobulin Gene Expression," *J. Exp. Med.*, 179:1585-1595 (1994).

Aucouturier, P.et al., "Monoclonal Ig L chain and L chain V domain fragment crystallization in myeloma-associated Fanconi's syndrome," *J. Immunol.*, 150(8):3561-3568 (1993).

Auerbach, et al., "Establishment and Chimera Analysis of 129/SvEv- and C57BL/6-Derived Mouse Embryonic Stem Cell Lines", *BioTechniques*, 29:1024-1032 (2000).

(Continued)

Primary Examiner — Scott Long

(74) *Attorney, Agent, or Firm* — Brownstein Hyatt Farber Schneck, LLP; Rita S. Wu; Yong-Jin Choi

(57) **ABSTRACT**

Genetically modified non-human animals are provided that comprise an immunoglobulin heavy chain locus comprising an unrearranged human heavy chain variable region nucleotide sequence comprising an addition of at least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon. Compositions and methods for making the genetically modified non-human animals as described herein are provided. Non-human animals capable of expressing an antigen-binding protein characterized by pH-dependent antigen binding, enhanced recyclability and/or enhanced serum half-life are also provided.

35 Claims, 19 Drawing Sheets

(56) **References Cited**

U.S. PATENT DOCUMENTS

2007/0280945	A1	12/2007	Stevens et al.	
2008/0069822	A1	3/2008	Jensen et al.	
2008/0078000	A1	3/2008	Poueymirou et al.	
2009/0035836	A1	2/2009	Datta et al.	
2009/0181855	A1	7/2009	Vasquez et al.	
2010/0069614	A1	3/2010	Houtzager et al.	
2010/0146647	A1	6/2010	Logtenberg et al.	
2010/0310586	A1	12/2010	Dolcetti et al.	
2010/0331527	A1	12/2010	Davis et al.	
2011/0076275	A1	3/2011	Igawa et al.	
2011/0098450	A1	4/2011	Igawa et al.	
2011/0111406	A1*	5/2011	Igawa et al.	435/6
2011/0195454	A1	8/2011	McWhirter et al.	
2011/0229489	A1*	9/2011	Pons et al.	424/158.1
2012/0021409	A1	1/2012	Mcwhirter et al.	
2012/0167237	A1	6/2012	Bradley et al.	
2012/0192300	A1	7/2012	Babb et al.	
2012/0204278	A1	8/2012	Bradley et al.	
2012/0322108	A1	12/2012	MacDonald et al.	
2013/0011866	A1	1/2013	Igawa et al.	
2013/0045492	A1	2/2013	Babb et al.	
2013/0145484	A1	6/2013	Logtenberg et al.	
2013/0185821	A1	7/2013	Babb et al.	
2013/0247236	A1*	9/2013	McWhirter et al.	800/18
2014/0013456	A1*	1/2014	McWhirter et al.	800/6

FOREIGN PATENT DOCUMENTS

CN	1560081	A	1/2005	
EP	1 317 537	A2	6/2003	
EP	1 439 234	A1	7/2004	
EP	1 605 058	B1	5/2009	
EP	2 147 594	A1	1/2010	
EP	2 275 443	A1	1/2011	
EP	2 427 357	A	3/2012	
EP	2 501 817	A	9/2012	
EP	2 505 654	A1	10/2012	
EP	2 517 556	A2	10/2012	
EP	2 517 557	A2	10/2012	
EP	2 556 747	A2	2/2013	
EP	2 564 695	A1	3/2013	
EP	2 582 230	A	4/2013	
EP	2762564	A1	8/2014	
WO	92/03918	A1	3/1992	
WO	94/02602	A1	2/1994	
WO	96/34096	A1	10/1996	
WO	98/46645	A2	10/1998	
WO	98/50431	A2	11/1998	
WO	02/20767	A2	3/2002	
WO	02/36789	A2	5/2002	
WO	2004/009618	A2	1/2004	
WO	2004/106375	A1	12/2004	
WO	2006/117699	A2	11/2006	
WO	2007/117410	A2	10/2007	
WO	2008/043822	A2	4/2008	
WO	2008/054606	A2	5/2008	
WO	2008/076379	A2	6/2008	
WO	2008/112922	A2	9/2008	
WO	WO2009/125825	*	10/2009	C07K 16/28
WO	2009/157771	A2	12/2009	
WO	2010/039900	A2	4/2010	
WO	2010/070263	A1	6/2010	
WO	2010/128897	A1	11/2010	
WO	2011/004192	A1	1/2011	
WO	2011/072204 AI		6/2011	
WO	2011/097603	A1	8/2011	
WO	2011097603	A1	8/2011	
WO	2011/111007	A2	9/2011	
WO	2011/122011	A2	10/2011	
WO	2011/158009	A1	12/2011	
WO	2011/163314	A1	12/2011	
WO	2012/141798	A1	10/2012	
WO	2012/148873	A2	11/2012	
WO	2013/022782	A1	2/2013	
WO	2013022782	A1	2/2013	
WO	2013/046722	A1	4/2013	
WO	2013/079953	A1	6/2013	
WO	2013/134263	A1	9/2013	
WO	2013/184761	A1	12/2013	
WO	2014/130690	A1	8/2014	

OTHER PUBLICATIONS

Basu, S.K., "Receptor-mediated endocytosis: An overview of a dynamic process," *J. Biosci.*, 6(4):535-542 (Aug. 6, 1984).

Bauer, S. et al., "Structure and pre-B lymphocyte restricted expression of the VpreB gene in humans and conservation of its structure in other mammalian species," *The EMBO Journal*, 7(1):111-116 (1988).

Beguinot, L. et al. "Down-regulation of the epidermal growth factor receptor in KB cells is due to receptor internalization and subsequent degradation in lysosomes," *Proc. Natl. Acad Sci. USA*, 81:2384-2388 (1984).

Brezinschek, H. et al., "Pairing of Variable Heavy and Variable κ Chains in Individual Naïve and Memory B Cells," *J. Immunol.*, 160(10):4762-4767 (1998).

Brown, M.S. et al., "Recycling Receptors: The Round-Trip Itinerary of Migrant Membrane Proteins," *Cell*, 22:663-667 (1983).

Carmack, C. et al., "Influence of a V kappa 8 L chain transgene on endogenous rearrangements and the immune response to the HA(Sb) determinant of influenza virus," *J. Immunol.*, 147(6):2024-2033 (1991).

Carter, P., "Bispecific human IgG by design," *Journal of Immunological Methods*, 248(1-2):7-15 (2001).

Cascalho, M. et al., "A Quasi-Monoclonal Mouse," *Science*, 272(5268):1649-1652 (1996).

Chaparro-Riggers, J. et al. "Increasing Serum Half-life and Extending Cholesterol Lowering in Vivo by Engineering Antibody with pH-sensitive Binding to PCSK9," *J. Biol. Chem.* 287(14):11090-11097 (2012).

Chinese Search Report for related Chinese Application No. 201180013714.0, mailed May 15, 2013.

Corbett, S.J. et al., "Sequence of the Human Immunoglobulin Diversity (D) Segment Locus: A Systematic Analysis Provides No Evidence for the Use of DIR Segments, Inverted D Segments, 'Minor' D Segments or D-D Recombination," *J. Mol. Biol.* 270:587-597 (1997).

Dall'Acqua W. F. et al., "Properties of Human IgG1s Engineered for Enhanced Binding to the Neonatal Fc Receptor (FcRn)," *J. Biol. Chem.*, 281:23514-23524 (2006).

Davies, et al., "Creation of Mice Expressing Human Antibody Light Chains by Introduction of a Yeast Artificial Chromosome Containing the Core Region of the Human Immunoglobulin κ Locus", *Nature Biotechnology*, 11:911-914, (1993).

de Kruif, J. et al., "Human Immunoglobulin Repertoires against Tetanus Toxoid Contain a Large and Diverse Fraction of High-Affinity Promiscuous VH Genes," *Journal of Molecular Biology*, 387:548-558 (2009).

de Wildt, R. et al., "Analysis of heavy and light chain pairings indicates that receptor editing shapes the human antibody repertoire," *J. Mol. Biol.*, 285(3):895-901 (1999).

Deng, R. et al. "Pharmacokinetics of Humanized Monoclonal Anti-Tumor Necrosis Factor-α Antibody and Its Neonatal Fc Receptor Variants in Mice and Cynomolgus Monkeys," *Drug Metabolism and Disposition*, 38(4):600-605 (2010).

Desienhofer, J., "Crystallographic Refinement and Atomic Models of a Human Fc Fragment and Its Complex with Fragment B of Protein A from *Staphylococcus aureus* at 2.9- and 2.8-Å Resolution," *Biochemistry*, 20(9):2361-2370 (1981).

Donohoe, M. et al., "Transgenic Human λ5 Rescues the Murine Lambda5 Nullizygous Phenotype," *Journal of Immunology*, 164:5269-5276 (2000).

Dunn, K.W. et al., "Iterative Fractionation of Recycling Receptors from Lysosomally Destined Ligands in an Early Sorting Endosome," *J. Cell. Biol.*, 109(6/2):3303-3314 (1989).

Edwards, D.R., et al., "The ADAM Metalloproteinases", *Molecular Aspects of Medicine*, 29(5): 258-289 (2008).

European Examination for Application No. 11 703 799.4 mailed Oct. 9, 2012.

(56) **References Cited**

OTHER PUBLICATIONS

European Communication for Application No. 12 173 456.0 mailed Dec. 5, 2012.
European Search Report for Application No. 12 173 456.0 dated Aug. 10, 2012.
Fallon, E.M. et al., "Increased Endosomal Sorting of Ligand to Recycling Enhances Potency of an Interleukin-2 Analog," *J. Biol. Chem.* 275(10):6790-6797 (2000).
Featherstone, K., et al., "The Mouse Immunoglobulin Heavy Chain V-D Intergenic Sequence Contains Insulators That May Regulate Ordered V(D)J Recombination", *The Journal of Biological Chemistry*, 285(13):9327-9338 (2010).
Festing, et al., "Revised nomenclature for strain 129 mice," *Mammalian Genome*, 10:836 (1999).
Fraenkel, S. et al., "Allelic 'choice' governs somatic hypermutation in vivo at the immunoglobulin kappa-chain locus," *Nat. Immunol.*, 8(7):715-722 (2007).
Gan, Z. et al.,"Analyses of the recycling receptor, FcRn, in live cells reveal novel pathways for lysosomal delivery," *Traffic*, 10(5):600. (2009).
Gay, D. et al., "Receptor editing: an approach by autoreactive B cells to escape tolerance," *J. Exp. Med.*, 177(4):999-1008 (1993).
Giallourakis, C.C., et al., "Elements between the IgH variable (V) and diversity (D) clusters influence antisense transcription and lineage-specific V(D)K recombination," *PNAS*, 107(51):22207-22212 (2010).
Goldstein, J.L. et al., "The LDL Receptor," *Arterioscler. Thromb. Vasc. Biol.*, 29:431-438 (2009).
Goletz et al., "Selection of Large Diversities of Antiidiotypic Antibody Fragments by Phage Display", *J. Mol. Biol.*, 315:1087-97, (2002).
Gonnet, et al., "Exhaustive Matching of the Entire Protein Sequence Database," *Science*, 256:1443-1445 (1992).
Gonzalez-Fernandez, A. et al., "Analysis of somatic hypermutation in mouse Peyer's patches using immunoglobulin κ light-chain transgenes," *PNAS USA*, 90:9862-9866 (1993).
Goyenechea, B. et al., "Modifying the sequence of an immunoglobulin V-gene alters the resulting pattern of hypermutation," *PNAS USA*, 93:13979-13984 (1996).
Green, L. et al., "Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs," *Nat. Genetics.*, 7(1):13-21 (1994).
Green, L. et al., "Regulation of B cell development by variable gene complexity in mice reconstituted with human immunoglobulin yeast artificial chromosomes," *J. Exp. Med.*, 188(3):483-495 (1998).
Han, C., et al., "Comprehensive Analysis of Reproductive ADAMs: Relationship of ADAM4 and ADAM6; with an ADAM Complex Required for Fertilization in Mice", *Biology of Reproduction*, 80(5): 1001-1008 (2009).
Hengstschlager, M. et al., "A λ1 transgene under the control of a heavy chain promoter and enhancer does not undergo somatic hypermutation," *Eur. J Immunol.*, 24:1649-1656 (1994).
Hendricks, J., et al., "Organization of the variable region of the immunoglobin heavy-chain gene locus of the rat," *Immunogenetics*, 62:479-486 (2010).
Hochedlinger, et al., "Monoclonal Mice Generated by Nuclear Transfer from Mature B and T Donor Cells", *Nature* 415(6875):1035-1038, (2002).
Igawa, T. et al., "Reduced elimination of IgG antibodies by engineering the variable region," *Protein Engineering, Design & Selection*, 23(5):385-392 (2010).
Igawa, T. et al., "Antibody recycling by engineered pH-dependent antigen binding improves the duration of antigen neutralization," *Nature Biotechnology*, 28(11):1203-1208 and supplement (2010).
Igawa T. et al, "Engineering the variable region of therapeutic IgG antibodies," mAbs, 3(3):243-52. (2011).
International Search Report and Written Opinion for International Patent Application No. PCT/US2011/023971 dated Apr. 11, 2011.
International Search Report and Written Opinion for International Patent Application No. PCT/US2012/034737 mailed Dec. 6, 2012.
International Search Report and Written Opinion for International Patent Application No. PCT/US2012/049600 mailed Nov. 23, 2012.
International Search Report and Written Opinion for International Patent Application No. PCT/US2013/029125 mailed Jun. 20, 2013.
International Search Report and Written Opinion for International Patent Application No. PCT/US2013/031834 mailed Jul. 2, 2013.
International Search Report and Written Opinion for International Patent Application No. PCT/US2013/032036 mailed Jul. 1, 2013.
International Search Report and Written Opinion for International Patent Application No. PCT/US2013/031823 mailed Jul. 8, 2013.
Ippolito, G.C., "Forced usage of positively charged amino acids in immunoglobulin CDR-H3 impairs B cell development and antibody production," *J. Exp. Med.*, 203(6):1567-1578 (2006).
Ito, W. et al., The His-probe method: effects of histidine residues introduced into the complementary-determining regions of antibodies on antigen-antibody interactions at different pH values, *FEBS Lett.*, 309(1):85-88. (1992).
Jakobovits, A. et al., "From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice," *Nature Biotechnology*, vol. 25, No. 10, pp. 1134-1143 (2007).
Jolly, C. et al., "Rapid methods for the analysis of immunoglobulin gene hypermutation: application to transgenic and gene targeted mice," *Nucleic Acids Research*, 25(10):1913-1919 (1997).
Kim, T., et al., "Expression and relationship of mail reproductive ADAMs in mouse," *Biology of Reproduction*, 74:744-750 (2006).
Klotz, E. et al., "Somatic Hypermutation of a λ2 Transgene Under the Control of the λ Enhancer or the Heavy Chain Intron Enhancer," *J. Immunol.*, 157:4458-4463 (1996).
Klotz, E. et al., "Somatic Hypermutation of an Artificial Test Substrate Within an lgκ Transgene," *J. Immunol.*, 161:782-790 (1998).
Kong, Q. et al., "A λ 3' enhancer drives active and untemplated somatic hypermutation of a λ_1 transgene," *J. Immunol.*, 161:294-301 (1998).
Kufer, P. et al., "A revival of bispecific antibodies," *Trends Biotechnol.*, 22(5):238-244 (May 2004).
Lee, E-C., et al., "The application of transgenic mice for therapeutic antibody discovery," *Methods in Molecular Biology*, 901:137-148 (2012).
Lencer, W. I. et al., "A passionate kiss, then run: exocytosis and recycling of IgG by FcRn," *Trends in Cell Biol.*, 15(1):5-9 (2005).
Lefranc, M., "Nomenclature of the Human Immunoglobulin Genes," *Current Protocols in Immunology*, Supplement 40, pp. A.1P.1-A.1P. 37 (2001).
Lefranc, et al. "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," *Dev. Comp. Immunol.*, 27:55-77 (2003).
Leitzgen, K. et al., "Assembly of immunoglobulin Light Chains as a Prerequisite for Secretion," *Journal of Biological Chemistry*, 272(5): 3117-3123 (1997).
Lindhofer, H. et al., "Preferential Species-Restricted Heavy/Light Chain Pairing in Rat/Mouse Quadromas," *The Journal of Immunology*, 155:219-225 (1995).
Lonberg et al., "Antigen-specific Human Antibodies from Mice Comprising Four Distinct Genetic Modifications", *Nature*, 368:856-859, (1994).
Lonberg, N., "Human antibodies from transgenic animals," *Nature Biotechnology*, 23(9):1117-1125 (2005).
Maeda, K. et al., "pH-dependent receptor/ligand dissociation as a determining factor for intracellular sorting of ligands for epidermal growth factor receptors in rat hepatocytes," *J. Controlled Release*, 82:71-82. (2002).
Marvin, J. et al., "Recombinant approaches to IgG-like bispecific antibodies," *Acta Pharmacologica Sinica*, 26(6):649-658 (2005).
Mellman, I., "The Importance of Being Acidic: The Role of Acidification in Intracellular Membrane Traffic," *J. Exp. Biol.*, 172:39-45 (1992).
Mendez, M. J. et al., "Functional Transplant of Megabase Human Immunoglobulin Loci Recapitulates Human Antibody Response in Mice," *Nat. Genetics*, 15(2):146-156 (1997).
Merchant, A. et al., "An efficient route to human bispecific IgG," *Nature Biotechnology*, 16(7):677-681 (1998).
Moran, Nuala "Mouse Platforms Jostle for Slice of Humanized Antibody Market", *Nature Biotech*, 3:267-268, (2013).

(56) **References Cited**

OTHER PUBLICATIONS

Murtaugh, M.L. et al., "A combinatorial histidine scanning library approach to engineer highly pH-dependent protein switches," *Protein Sci.*, 20(9):1619-1631 (2011).

Nakasako, M. et al., "The pH-dependent Structural Variation of Complementarity-determining Region H3 in the Crystal Structures of the Fv Fragment from an Anti-dansyl Monoclonal Antibody," *J. Mol. Biol.*, 291:117-134 (1999).

Nicholson, I. et al., "Antibody Repertoires of Four- and Five-Feature Translocus Mice Carrying Human Immunoglobulin Heavy Chain and κ and λ Light Chain Yeast Artificial Chromosomes," *Journal of Immunology*, 163:6898-6906 (1999).

O'Brien, R. et al., "Somatic hypermutation of an immunoglobulin transgene in κ mice," *Nature*, 326(6111):405-409 (1987).

Pelanda, R. et al., "A prematurely Expressed Igκ Transgene, but Not VκJκ Gene Aegment Targeted into the Igκ Locus, Can Rescue B Cell Development in λ5-Deficient Mice," *Immunity*, 5(3):229-239 (1996).

Petkova, S.B. et al., "Enhanced half-life of genetically engineered human IgG1 antibodies in a humanized FcRn mouse model: potential application in humorally mediated autoimmune disease," *Intl. Immunol.*, 18(12):1759-1769 (2006).

Poueymirou, W. et al. F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses, *Nature Biotech.*, 25(1):91-99 (2007).

Prak, E. et al., "Light chain replacement: a new model for antibody gene rearrangement," *J. Exp. Med.*, 182(2):541-548 (1995).

Presta, L.G., "Molecular engineering and design of therapeutic antibodies," *Curr. Opin. Immunol.*, 20(4):460-470 (2008).

Raso, V. et al., "Intracellular Targeting with Low pH-triggered Bispecific Antibodies," *J Biol. Chem.*, 272(44):27623-27628 (1997).

Roberts, D.M. et al., "Isolation and Characterization of the Fc Receptor from Fetal Yolk Sac of the Rat," *J Cell. Biol.*, 111:1867-1876 (1990).

Rojas, G. et al., "Phage antibody fragments library combining a single human light chain variable region with immune mouse heavy chain variable regions," *Journal of Biotechnology*, 94:287-298 (2002).

Roopenian, D.C, et al., "FcRn: the neonatal Fc receptor comes of age," *Nature Rev. Immunol.*, 7:715-725 (Sep. 2007).

Roopenian, D.C., et al., "Clinical Ramifications of the MHC Family Fc Receptor FcRn," *J. Clin. Immunol.*, 30(6):790-797 (2010).

Sarkar, C.A. et al. "Rational cytokine design for increased lifetime and enhanced potency using pH-activated 'histidine switching'," *Nature Biotech.*, 20:908-913 (2002).

Schroeder, H.W., et al., "Similarity and divergence in the development and expression of the mouse and human antibody repertoires," *Developmental and Comparative Immunol.*, 30(1-2):119-135 (2006).

Seals, D.F., et al., "the ADAMs family of metalloproteases: multidomain; proteins with multiple functions," *Genes and Development*, 17(1):7-30 (2003).

Simister, Neil E., et al. An Fc receptor structurally related to MHC class I antigens, *Nature* 337:184-187 (Jan. 12, 1989).

Sirac, C. et al., "Role of the monoclonal kappa chain V domain and reversibility of renal damage in a transgenic model of acquired Fanconi syndrome," *Blood*, 108(2):536-543 (2006).

Smith, B. et al., "The unique and immunoglobulin-like regions of surrogate light chain component lambda5 differentially interrogate immunoglobulin heavy-chain structure," *Molecular Immunology*, 47:1195-1206 (2010).

Storb, et al., "Transgenic Mice with μ and κ Genes Encoding Antiphosphorycholine Antibodies", *J. Exp Med*, 164:627-641 (1986).

Suzuki, T. et al., "Importance of Neonatal FcR in REgulation the Serum Half-Life of Therapeutic Proteins Containing the Fc Domain of Human IgG1: A Comparative Study of the Affinity of Monoclonal Antibodies and Fc-Fusion Proteins to Human Neonatal FcR," *J. Immunol.*, 184:1968-1976 (2010).

Reply to Third Party Observations on European patent application 11 703 799.04 (Publication No. EP 2 501 817) filed in EPO on May 20, 2013.

Request to provoke an interference U.S. Appl. No. 13/750,753 Jan. 25, 2013.

Summons to attend oral proceedings arranged in connection with European patent application 09075279.1 (Publication No. EP 2 147 594 A1) mailed Mar. 6, 2013.

Tabrizi, M. A. et al. "Elimination mechanisms of therapeutic monoclonal antibodies," *Drugs Discovery Today*, 11(1/2):81-88 (2006).

Taylor et al., "A Transgenic Mouse that Expresses a Diversity of Human Sequence Heavy and Light Chain Immunoglobulins," *Nucleic Acid Research*, 20(23):6287-6295 (1992).

Taylor et al., "Human immunoglobulin transgenes undergo rearrangement, somatic mutation and class switching in mice that lack endogenous 1gM", *Int. Immunol.*, 6:579-591 (1994).

Third Party Observations Under Article 115 EPC against European Parent Application No. 09075279.1 filed in EPO on Oct. 25, 2012.

Third Party Observations on European patent application 11 703 799.4-2405 (Publication No. EP 2 501 817) mailed on Feb. 28, 2013.

Tsubata, T. et al., "The Products of the Pre-B Cell-specific Genes(Lambda5 and VpreB) and the Immunoglobulin mu Chain Form a Comples that is Transported onto the Cell Surface," *Journal of Experimental Medicine*, 172:973-976 (1990).

Tuaillon, et al., "Analysis of direct and inverted DJH rearrangements in a human Ig heavy chain transgenic minilocus," *Journal of Immunology.*, 154(12):6453-6465 (1995).

Tutt, A. et al., "Trispecific F(ab')3 Derivatives That Use Cooperative Signaling Via the TCR/CD3 Complex and CD2 to Activate and Redirect Resting Cytotoxic T Cells," *Journal of Immunology*, 147(1):60-69 (Jul. 1, 1991).

Tzaban, S. et al., "The recycling and transcytotic pathways for IgG transport by FeRn are distinct and display an inherent polarity," *J. Cell Biol.*, 185(4):673-684 (2009).

Valenzuela, D. M. et al., "High-throughput engineering of the mouse genome coupled with high-resolution expression analysis," *Nature Biotech.*, 21(6):652-659 (2003).

Vaughn, D.E., et al., "Structural basis of pH-dependent antibody binding by the neonatal receptor," *Structure*, 6:63-73(1997).

Wang, W. et al. "Monoclonal Antibody Pharmacokinetics and Pharmacodynamics," *Clinical Pharmacology & Therapeutics*, 84(5):548-558 (2008).

Watanabe, H. et al. "Optimizing pH Response of Affinity between Protein G and IgG Fc: How Electrostatic Modulations Affect Protein-Protein Interactions," *J. Biol. Chem.*, 284(18):12373-12383 (2009).

Xu, L. et al., "Combinatorial surrobody libraries," *Proceedings of the National Academy of Sciences (USA)*, 105(31):10756-10761 (2008).

Yeung, Y.A. et al. "Engineering Human IgG1 Affinity to Human Neonatal Fc Receptor: Impact of Affinity Improvement on Pharmacokinetics in Primates," *J. Immunol.*, 182(12):7663-7671 (2009).

U.S. Non-Final Office Action for U.S. Appl. No. 13/022,759 mailed Sep. 7, 2012.

U.S. Non-Final Office Action for U.S. Appl. No. 13/093,156 mailed Sep. 6, 2012.

U.S. Non-Final Office Action for U.S. Appl. No. 13/412,936 mailed Sep. 6, 2012.

International Search Report and Written Opinion mailed Sep. 4, 2013, from related International Patent Application No. PCT/US2013/044257 filed Jun. 5, 2013.

Aucouturier et al., (1992) "Human rearranged IgK mRNA VJC region," GenBank Accession No. M87478 1 page, first referenced Mar. 3, 1992, first seen at NCBI Apr. 27, 1993.

Choi et al., (2004) "Characterization and comparative genomic analysis of intronless Adams with testicular gene expression," Genomics 83(4): 636-646.

Dechiara et al., (2009) Chapter 16: VelociMouse: Fully ES Cell-Derived FO Generation Mice Obtained from the Injection of ES Cells into 8-Cell Stage Embryos, Gene Knockout Protocols: Second Edition, vol. 530, Humana Press.

(56) **References Cited**

OTHER PUBLICATIONS

Fishwild et al., (1996) "High-avidity human IgGk monoclonal antibodies from a novel train of mililocus transgenic mice," Nature Biotechnology, 14(7):845-851.
Goodhardt et al., (1987), "Rearrangement and Expression of rabbit immunoglobulin K light chain gene in transgenic mice," PNAS, 84:4229-4233.
Goyenechea et al., (1997) "Cells strongly expressing Ig(kappa) transgenes show clonal recruitment of hypermutation: a role for both MAR and the enhancers," EMBO J., 16(13):3987-94.
Green et al., (1999) "Antibody engineering via geneitc engineering of the mouse: XenoMouse strains are a vehicle for the facile generation of therapeutic human monoclonal antibodies," J. Immunol. Methods, 231:11-23.
Hardy and Hayakawa, (2001) "B cell development pathways," Annu. Rev. Immunol., 19:595-621.
Hömig-Hölzel et al., (2008) "Constitutive CD40 signaling in B cells selectively activates the noncanonical NF-kappaB pathway and promotes lymphomagenesis," J. Exp. Med., 205(6):1317-1329.
Inlay et al., (2002) "Essential roles of the kappa light chain intronic enhancer and 3' enhancer in kappa rearrangement and demethylation," Nat. Immunol., 3(5):463-468.
Jakobovits, (1995) "Production of fully human antibodies by transgenic mice," Curr. Opin. Biotechnol., 6 (5):561-566.
Janeway's Immunobiology, (2008) Seventh Edition, Murphy, Travers and Walpot, eds., Garland Science, New York and London, Ch. 4, pp. 145-155.
Kabat and Wu, (1991) "Identical V region amino acid sequences and segments of sequences in antibodies of different specificities. Relative contributions of VH and VL genes, minigenes, and complementarity-determining regions to binding of antibody-combining sites," J. Immunol., 147(5):1709-1719.
Kaushik, (1990) "Stochastic pairing of heavy-chain and x light-chain variable gene families occurs in polyclonally activated B cells," Proc. Natl. Acad. Sci. USA, 87: 4932-4936.
Klöhn et al., (2013) "Ibc's 23rd Annual Antibody Engineering, 10th Annual Antibody Therapeutics international conferences and the 2012 Annual Meeting of The Antibody Society: Dec. 3-6, 2012," San Diego, CA, Mabs, 5 (2):178-201.
Logtenberg, (2007) "Antibody cocktails: next-generation biopharmaceuticals with improved potency," Trends Biotechnol., 25(9):390-394.
Nagle, (2007) "Regeneron helps make Sanofi Veloclmmune to its "weak pipeline,"" <http://www.outsourcing-pharma.com> —Published Dec. 3, 2007.
Nemazee, (2006) "Receptor editing in lymphocyte development and central tolerance," Nat. Rev. Immunol., 6 (10):728-740.
News in Brief Article (2007) "Big Pharma vies for mice," Nature Biotechnology, 25(6):613—Published Jun. 2007.
No Author Listed, Additional post-filing data and letter filed by the Applicant/Patentee for corresponding European application 09075279.1, now opposed patent EP 2147594 B1, 4 pages (Jun. 13, 2013).
No Author Listed, "Next generation transgenic mice for therapeutic human antibodies, Description of MeMoTM," filed by the Applicant/Patentee in prosecution for corresponding European application 09075279.1, now opposed patent EP 2147594 B1, 2 pages (Dec. 22, 2011).
No Author Listed, (2011) Chapter 8: The Development and Survival of Lymphocytes, Janeway's Immunobiology, 8th Edition, Eds. Kenneth Murphy et al, Garland Science (ISBN: 9780815342434), whole document, in particular p. 279 and Figure 8.4.
Orban et al., (1992) "Tissue- and site-specific DNA recombination in transgenic mice," Proc. Natl. Acad. Sci. U S A., 89(15):6861-6865.
Panka et al., (May 1988) "Variable region framework differences result in decreased or increased affinity of variant anti-digoxin antibodies," Proc. Natl. Acad. Sci. USA, 85:3080-3084.
Paul, (1993) Fundamental Immunology, 3rd ed., Raven Press, NY, Chapter 9, pp. 292-295.
Popov et al., (1999) "A human immunoglobulin lambda locus is similarly well expressed in mice and humans," J. Exp. Med., 189(10):1611-1620.
Rabquer et al., (2005) "Immunoglobulin light chain variable region, partial [Homo sapiens]," GenBank Accession No. ABA26122, 2 pages, first reference Dec. 31, 1995.
Rickert et al., (1997) "B lymphocyte-specific, Cre-mediated mutagenesis in mice," Nucleic Acids Res., 25 (6):1317-1318.
Rudikoff et al., (1982) "Single amino acid substitution altering antigen-binding specificity," Proc. Natl. Acad. Sci. USA, 79:1979-1983.
Sasaki et al., (2006) "Canonical NF-x13 Activity, Dispensable for B Cell Development, Replaces BAFF-Receptor Signals and Promotes B Cell Proliferation upon Activation," Immunity, 24:729-739.
Scott, (2007) "Mice with a human touch," Nature Biotechnology, 25(10): 1075-1077.
Sharpe et al., (1991) "Somatic hypermutation of immunoglobulin kappa may depend on sequences 3' of C kappa and occurs on passenger transgenes," EMBO J., 10(8):2139-2145.
Simon and Rajewsky, (1990), "Antibody domain mutants demonstrate autonomy of the antigen binding site," EMBO J., 9(4):1051-1056.
Soriano, (1999) "Generalized lacZ expression with the ROSA26 Cre reporter strain," Nat. Genet., 21(1):70-71.
Stevens et al., (2008) "Human Antibody Discovery, Veloclmmune—A novel platform, Pharma Focus Asia," Issue 8:72-74.
Torres and Kuhn, (1997) "Laboratory Protocols for Conditional Gene Targeting," Oxford University Press, 978-0-19-963677-8, 42-53.
Vaughan et al., (1996) "Human antibodies with sub-nanomolar affinities isolated from a large non-immunized phage display library," Nat. Biotechnol., 14(3):309-314.
Winter et al., (1997) "Insertion of 2 kb of bacteriophage DNA between an immunoglobulin promoter and leader exon stops somatic hypermutaion in a kappa transgene," Mol. Immunol., 34(5):359-366.
International Search Report and Written Opinion for PCT Application No. PCT/US2013/044257 mailed Sep. 4, 2014 (9 pages).
International Search Report and Written Opinion for PCT Application No. PCT/US2014/025982 mailed Jul. 22, 2014 (13 pages).
International Search Report and Written Opinion for PCT Application No. PCT/US2014/026040 mailed Jul. 29, 2014, (14 pages).
International Search Report and Written Opinion for PCT Application No. PCT/US2014/056285 mailed Feb. 2, 2015, (12 pages).
Sirac et al. (2011) "Toward Understanding Renal Fanconi Syndrome: Step by Step Advances through Experimental ModeLs," Exp. Models for Renal Diseases: Pathogenesis and Diagnosis, Contrib. Nephrol. Basel, Karger, 169:247-261.
Statement of Relatedness under MPEP 2001.06 dated Oct. 14, 2015.

* cited by examiner

	Stop	SEQ ID NO.	Hydrophilic	SEQ ID NO.	Hydrophobic	SEQ ID NO.
D1-1	VQLER	8	YNWND	45	GTTGT	88
HD1-1	VPLAR	9	YHWHD	46	GTTGT	88
D1-7	V*LEL	-	YNWNY	47	GITGT	89
HD1-7	VSLAL	10	YHWHY	48	GITGT	89
D1-20	V*LER	-	YNWND	45	GITGT	89
HD1-20	VSLAR	11	YHWHD	46	GITGT	89
D1-26	V*WELL	12	YSGSYY	49	GIVGAT	90
HD1-26	VSWEPL	13	YHGSHY	50	GIMGAT	91
D2-2*02	RIL**YQLLY	14	GYCSSTSCYT	51	DIVVVPAAI	92
HD2-2*02	RTL*SYQLPY	15	GHCSHTSCHT	52	DIVVIPAAI	93
D2-8*01	RILY*WCMLY	16, 17	GYCTNGVCYT	53	DIVLMVYAI	94
HD2-8*01	RTLYSWCMPY	18	GHCTHGVCHT	54	DIVLMVYAI	94
D2-15	RIL*WW*LLL	-	GYCSGGSCYS	55	DIVVVVAAT	95
HD2-15	RTL*SW*LPL	-	GHCSHGSCHS	56	DIVVMVAAT	96
D2-21*02	SILWW*LLF	19	AYCGGDCYS	57	HIVVVTAI	97
HD2-21*02	STLWWSLPF	20	AHCGGHCHS	58	HIVVVTAI	97
D3-3*01	VLRFLEWLLY	21	YYDFWSGYYT	59	ITIFGVVII	98
HD3-3*01	VSPFLEWSLY	22	YHHFWSGHYT	60	ITIFGVVII	98
D3-9	VLRYFDWLL*	23	YYDILTGYYN	61	ITIF*LVII	99, 100
HD3-9	VSPYFDWSL*	24	YHHILTGHYN	62	ITIF*LVII	99, 100
D3-10*01	VLLWFGELL*	25	YYYGSGSYYN	63	ITMVRGVII	101
HD3-10*01	VSPWFGESL*	26	YHHGSGSHYN	64	ITMVRGVII	101
D3-16*02	VL*LRLGELSLY	27	YYDYVWGSYRYT	65	IMITFGGVIVI	102
HD3-16*02	VS*SRLGESSLY	28	YHDHVWGSHRYT	66	IMITFGGVIVI	102
D3-22	VLL***WLLL	29	YYYDSSGYYY	67	ITMVVVIT	103
HD3-22	VSLS**WSLL	30, 31	YHYHSSGHYY	68	ITIVVVIT	104
D4-4	*LQ*L	-	DYSNY	69	TTVT	105
HD4-4	*PQSL	32	DHSHY	70	TTVT	105
D4-11p	*LQ*L	-	DYSNY	69	TTVT	105

FIG. 1A

	Stop	SEQ ID NO.	Hydrophilic	SEQ ID NO.	Hydrophobic	SEQ ID NO.
HD4-11p	*PQSL	32	DHSHY	70	TTVT	105
D4-17	*LR*L	-	DYGDY	71	TTVT	105
HD4-17	*PRSL	33	DHGHY	72	TTVT	105
D4-23p	*LRW*L	-	DYGGNS	73	TTVVT	106
HD4-23p	*PRWSL	34	DHGGHS	74	TTVVT	106
D5-5	WIQLWL	35	GYSYGY	75	VDTAMV	107
HD5-5	WTQPWL	36	GHSHGY	76	VDTAMV	107
D5-12	WI*WLRL	37	GYSGYDY	77	VDIVATI	108
HD5-12	WT*WPPL	38	GHSGHHY	78	VDIVATI	108
D5-18	WIQLWL	35	GYSYGY	75	VDTAMV	107
HD5-18	WTQPWL	36	GHSHGY	76	VDTAMV	107
D5-24p	*RWLQL	39	RDGYNY	79	VEMATI	109
HD5-24p	*TWPPL	40	RHGHHY	80	VDMATI	110
D6-6	V*QLV	-	EYSSSS	81	SIAAR	111
HD6-6	A*PLV	-	EHSHSS	82	SIATR	112
D6-13	V*QQLV	41	GYSSSWY	83	GIAAAG	113
HD6-13	A*PQLV	42	GHSHSWY	84	GIATAG	114
D6-19	V*QWLV	43	GYSSGWY	85	GIAVAG	115
HD6-19	A*PWLV	44	GHSHGWY	86	GIAMAG	116
D6-25	V*QRL	-	GYSSGY	87	GIAAA	117
HD6-25	A*PRL	-	GHSHGY	76	GIATA	118

FIG. 1B

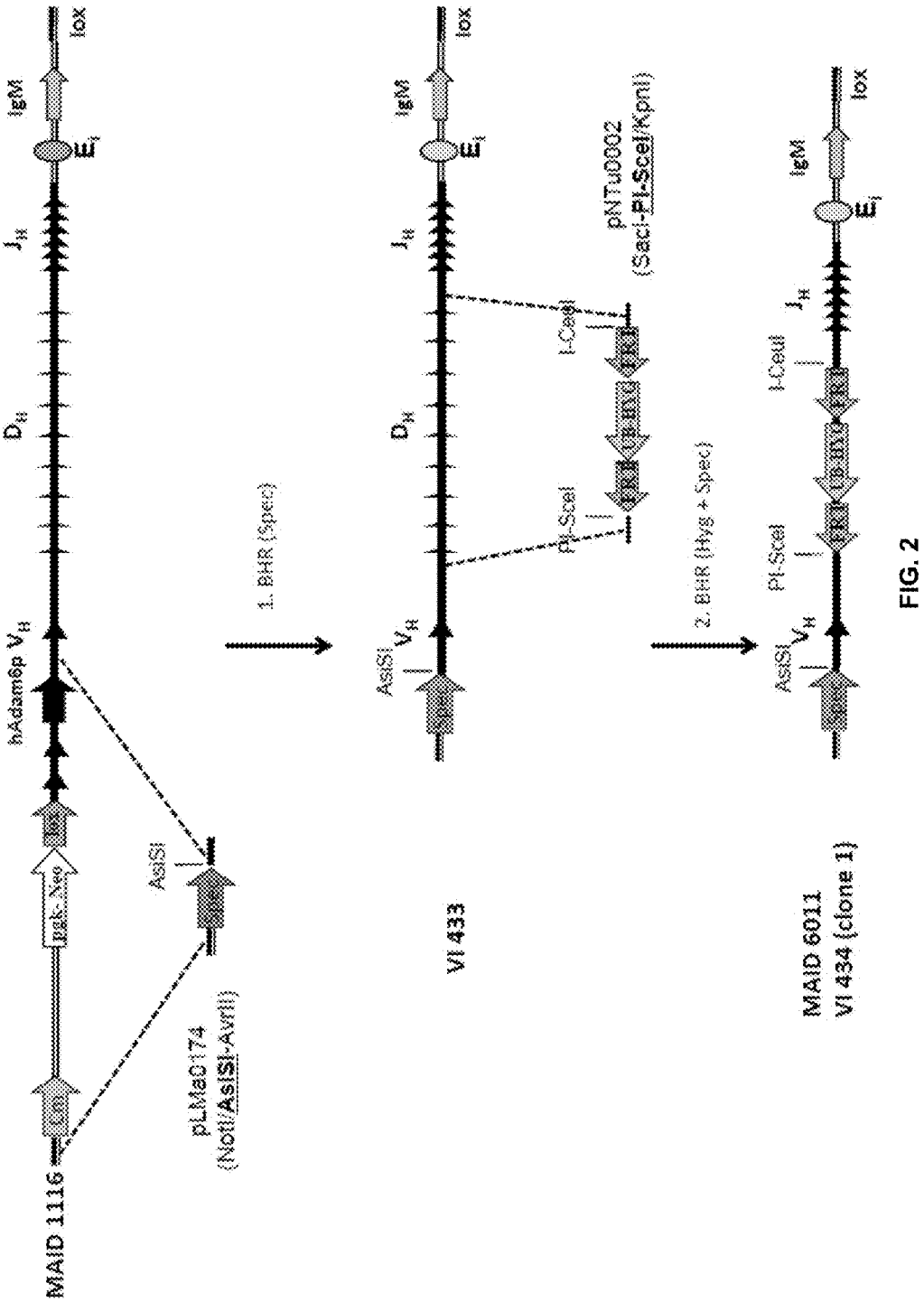

MAID 1116
pLMa0174
(NotI/AsiSI-AvrII)
AsiSI
Ub
Bsd-Neo
hAdam6p
V_H
D_H
J_H
IgM
lox
1. BHR (Spec)
VI 433
AsiSI
I-CeuI
PI-SceI
pNTu0002
(SacI-PI-SceI/KpnI)
2. BHR (Hyg + Spec)
MAID 6011
VI 434 (clone 1)

FIG. 2

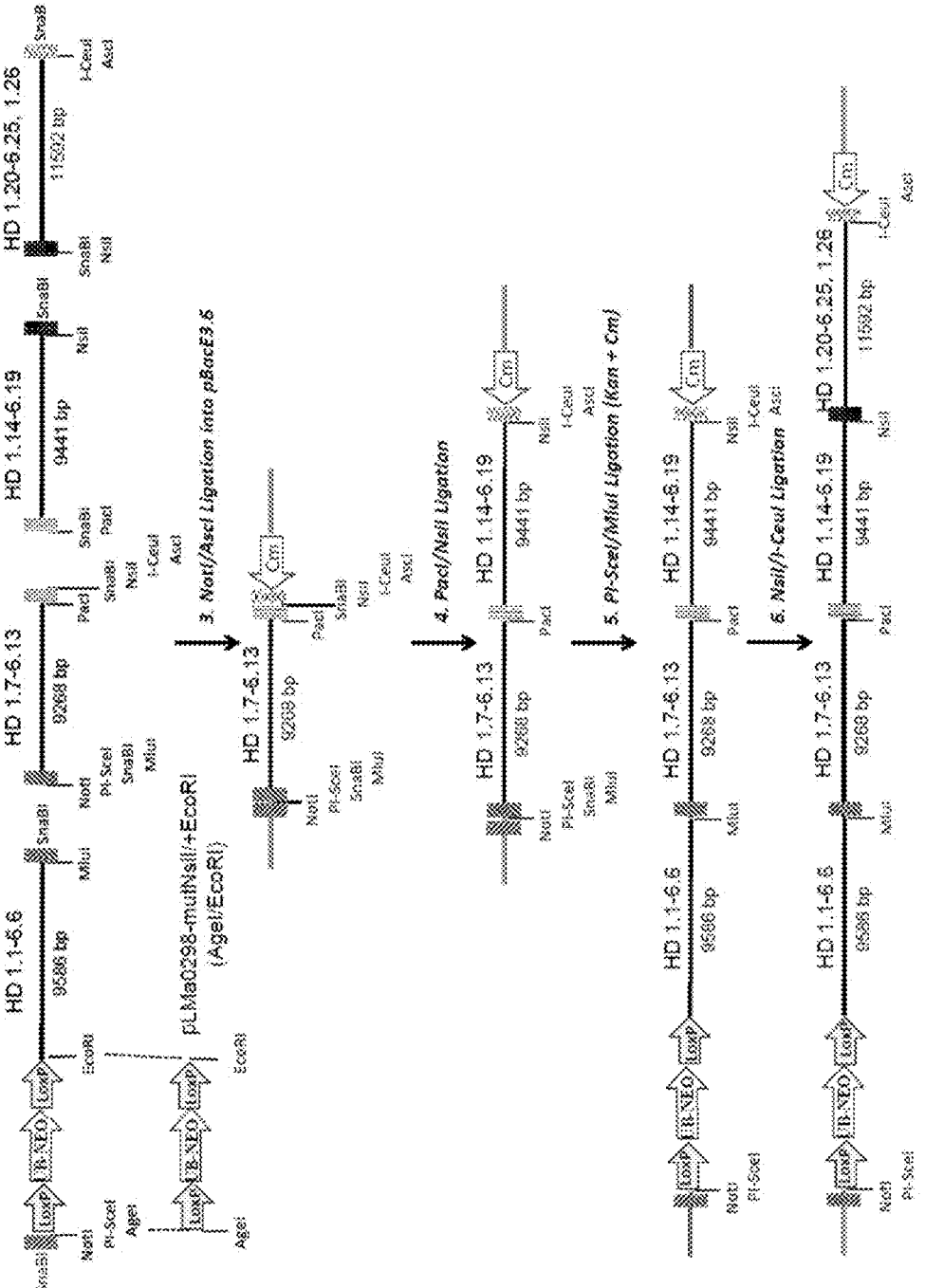

HD 1.1-6.6
9586 bp
HD 1.7-6.13
9268 bp
HD 1.14-6.19
9441 bp
HD 1.20-6.25, 1.26
11592 bp
pLMa0298-mutNsiI/+EcoRI
(AgeI/EcoRI)
3. NotI/AscI Ligation into pBACE3.6
4. PacI/NsiI Ligation
5. PI-SceI/MluI Ligation (Kan + Cm)
6. NsiI/I-CeuI Ligation
Cm
TB-NEO
LoxP

FIG. 3

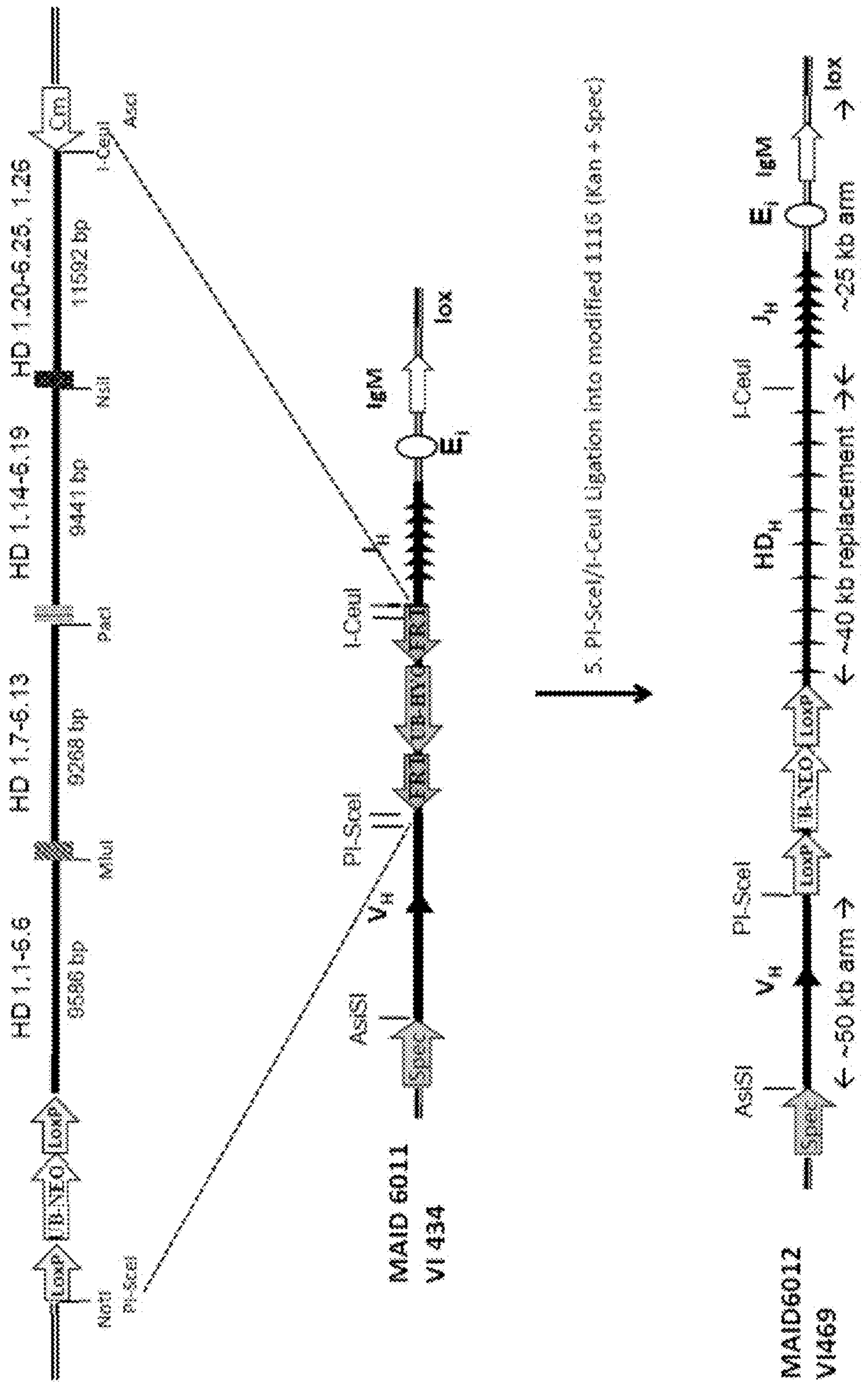

HD 1.1-6.6
9596 bp
HD 1.7-6.13
9268 bp
HD 1.14-6.19
9441 bp
HD 1.20-6.25, 1.26
11592 bp
NotI
PI-SceI
MluI
PacI
NsiI
I-CeuI
AscI
Cm
MAID 6011
VI 434
AsiSI
PI-SceI
I-CeuI
lgM
lox
5. PI-SceI/I-CeuI Ligation into modified 1115 (Kan + Spec)
MAID6012
VI469
AsiSI
PI-SceI
I-CeuI
HD_H
lgM
lox
← ~50 kb arm →
← ~40 kb replacement →
← ~25 kb arm →

FIG. 4

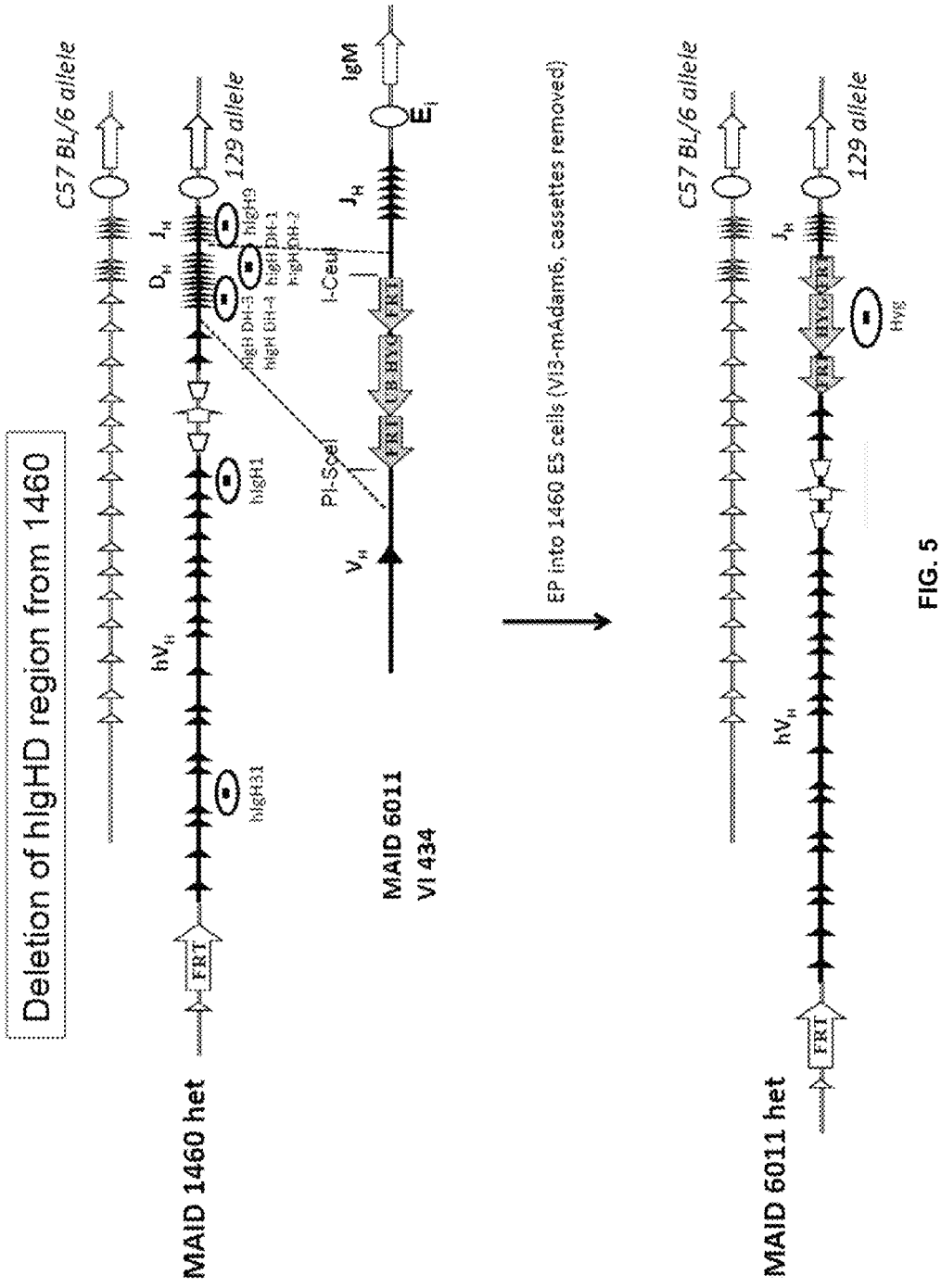

Deletion of hIgHD region from 1460
C57 BL/6 allele
129 allele
MAID 1460 het
MAID 6011
VI 434
EP into 1460 ES cells (VI3-mAdam6, cassettes removed)
C57 BL/6 allele
129 allele
MAID 6011 het
IgM
FRT
Hyg

FIG. 5

Probes for MAID 6011 (deletion of higH DH segments in MAID1460)

Name	Forward Primer	Probe	Reverse Primer	Type	Label	Location
higH DH-1	CGGGTCACTGCCATTTCTG (SEQ ID NO: 119)	TCTGCATTCGCTCCCAGCGC (SEQ ID NO: 120)	TCTGCGGCATGAACCCAAT (SEQ ID NO: 121)	LOA	FAM-BHQ 1	higH D segments
higH DH-2	GTGCAGGGAGGACCTTCT G (SEQ ID NO: 122)	AGTCACCAAGCACAGAGCCCTGAC (SEQ ID NO: 123)	GCCAGGGAGTTGCCTAGTG (SEQ ID NO: 124)	LOA	FAM-BHQ 1	higH D segments
higH DH-3	GTGGCCCACTTCCCTTCCT (SEQ ID NO: 125)	CAGCTGGAACCCACCATGACCT (SEQ ID NO: 126)	GACCTGCCTCTGGATGACA (SEQ ID NO: 127)	LOA	FAM-BHQ 1	higH D segments
higH DH-4	TGCCCAGAACTGACCCTAC (SEQ ID NO: 128)	ACCGACAAGAGTCCCTCAGG (SEQ ID NO: 129)	GGAGTCGGCTCTGGATGTG (SEQ ID NO: 130)	LOA	BHQ-plus	higH D segments
hyg	TGCGGCCGATCTTAGCC (SEQ ID NO: 131)	ACGAGCGGGTTCGGCCCATTC (SEQ ID NO: 132)	TTGACCGATTCCTTGCGG (SEQ ID NO: 133)	GOA	FA-BHQ 1	
higH1	CAGTCCCGTTGATCCAGCC (SEQ ID NO: 134)	CCCATCAGGGATTTTGTATCTCTGT GGACG (SEQ ID NO: 135)	GGATATGCAGCACTGTGCTAC (SEQ ID NO: 136)	AR		higH
higH9	TCCTCCAACGACAGGTCCC (SEQ ID NO: 137)	TCCCTGGAACTCTGCCCCGACACA (SEQ ID NO: 138)	GATGAACTGACGGGCACAGG (SEQ ID NO: 139)	AR		higH
higH31	ATCACACTCATCCCATCCCC (SEQ ID NO: 140)	CCCTTCCCTAAGTACCACAGAGTGG GCTC (SEQ ID NO: 141)	CACAGGGAAGCAGGAACTGC (SEQ ID NO: 142)	AR		higH

FIG. 6

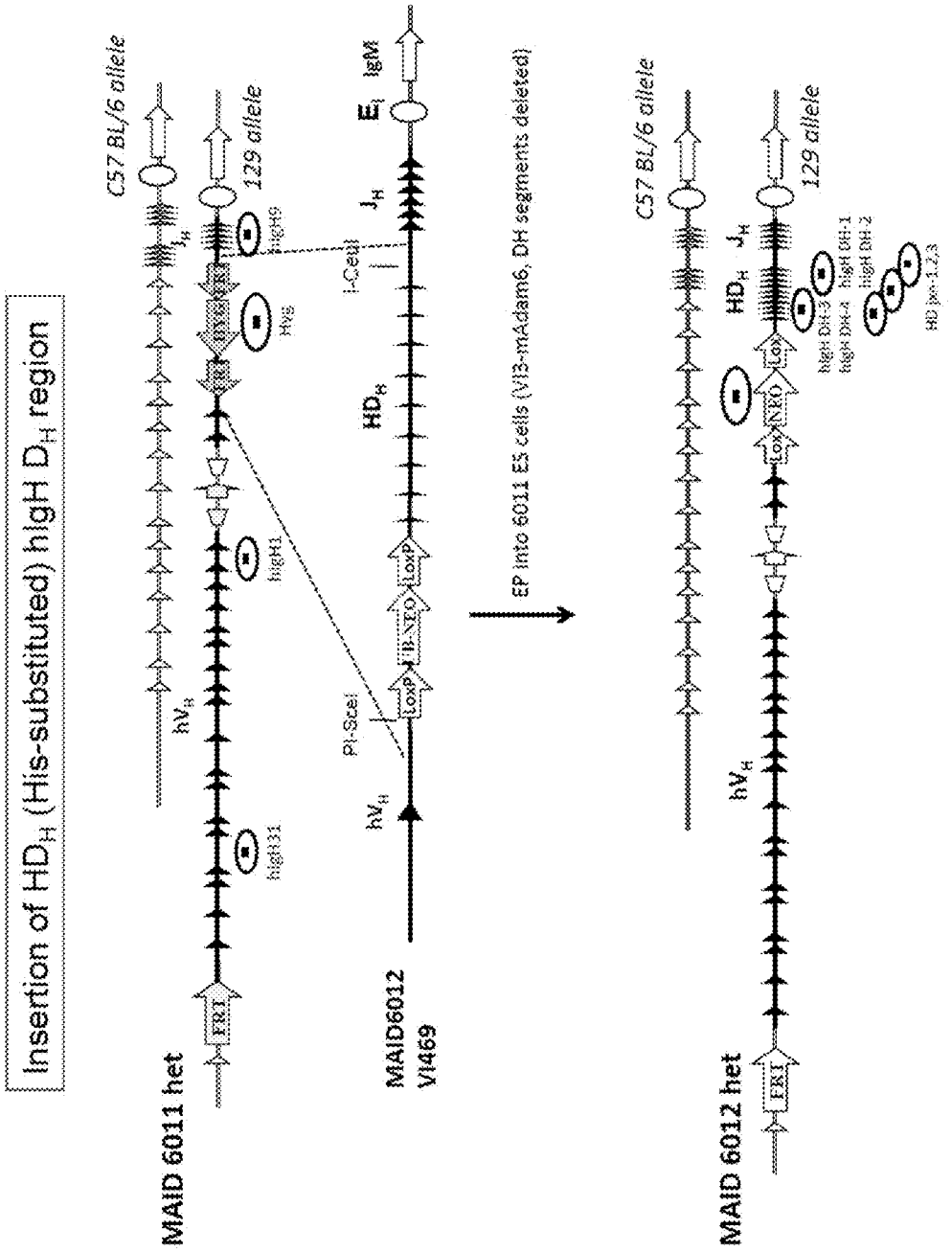

Insertion of HDH (His-substituted) hIgH DH region
C57 BL/6 allele
129 allele
MAID 6011 het
MAID6012
VI469
I-CeuI
PI-SceI
EP into 6011 ES cells (V13-mAdam6, DH segments deleted)
MAID 6012 het

FIG. 7

Probes for MAID 6012 (insertion of HD, His-substituted higH DH segments)

Name	Forward Primer	Probe	Reverse Primer	Type	Label	Location
hyg	TGCGGCCGATCTTAGCC (SEQ ID NO: 131)	ACGAGCGGGTTCGGCCCATTC (SEQ ID NO: 132)	TTGACCGATTCCTTGCGG (SEQ ID NO: 133)	LOA	FAM-BHQ1	
HD jxn-1	GGAGCCAGGCAGGACACA (SEQ ID NO: 143)	TGGGCTCGTAGTTTGACGT (SEQ ID NO: 144)	GGGACTTTCTTACCCACA CTTCA (SEQ ID NO: 145)	GOA	MGB	Synthetic linker-1 in HD segments
HD jxn-2	GGTCCCGAGCACTCTTAATTAAA C (SEQ ID NO: 146)	CCTCGAATGGAACTAC (SEQ ID NO: 147)	GGGAGAGCAACCATTCG TTGT (SEQ ID NO: 148)	GOA	MGB	Synthetic linker-2 in HD segments
HD jxn-3	CCGAGCACCGATGCATCTA (SEQ ID NO: 149)	CGCAGTCATGTAATGC (SEQ ID NO: 150)	GGGAGGCGAACTGACTG TCA (SEQ ID NO: 151)	GOA	MGB	Synthetic linker-3 in HD segments
higH DH-1	CGGGTCACTGCCATTCTG (SEQ ID NO: 119)	TCTGCATTCGCTCCCAGCGC (SEQ ID NO: 120)	TCTGCGGCATGAACCCAA T (SEQ ID NO: 121)	GOA	FAM-BHQ1	higH D segments
higH DH-2	GTGCAGGGAGGACCTTCTG (SEQ ID NO: 122)	AGTCACCAAGCACAGAGCCCTGA C (SEQ ID NO: 123)	GCCAGGGAGTTGCCTAG TG (SEQ ID NO: 124)	GOA	FAM-BHQ1	higH D segments
higH DH-3	GTGGCCCACTTCCCTTCCT (SEQ ID NO: 125)	CAGCTGGGAACCCACCATGACCT (SEQ ID NO: 126)	GACCTGCCTCGGATGACA (SEQ ID NO: 127)	GOA	FAM-BHQ1	higH D segments
higH DH-4	TGGCCAGAACTGACCCTAC (SEQ ID NO: 128)	ACCGACAAGAGTCCCTCAGG (SEQ ID NO: 129)	GGAGTCGGCTCTGGATGTG (SEQ ID NO: 130)	GOA	BHQ-plus	higH D segments
neo	GGTGGAGAGGCTATTCGGC (SEQ ID NO: 152)	TGGGCACAACAGACAATCGGCTG (SEQ ID NO: 153)	GAACACGGGCGGCATCAG (SEQ ID NO: 154)	GOA	FAM-BHQ1	
higH1	CAGTCCGTTGATCCAGCC (SEQ ID NO: 134)	CCCATCAGGGATTTTGTATCTC TGTGGACG (SEQ ID NO: 135)	GGATATGCAGCACTGTGCC AC (SEQ ID NO: 136)	AR		higH
higH9	TCCTCCAACGACAGGTCCC (SEQ ID NO: 137)	TCCCTGGAACTCTGCCCCGACACA (SEQ ID NO: 138)	GATGAACTGACGGGGCACA GG (SEQ ID NO: 139)	AR		higH
higH31	ATCACACTCATCCCATCCCC (SEQ ID NO: 140)	CCCTTCCCTAAGTACCACAGAGTG GGCTC (SEQ ID NO: 141)	CACAGGGAAGCAGGAACT GC (SEQ ID NO: 142)	AR		higH

FIG. 8

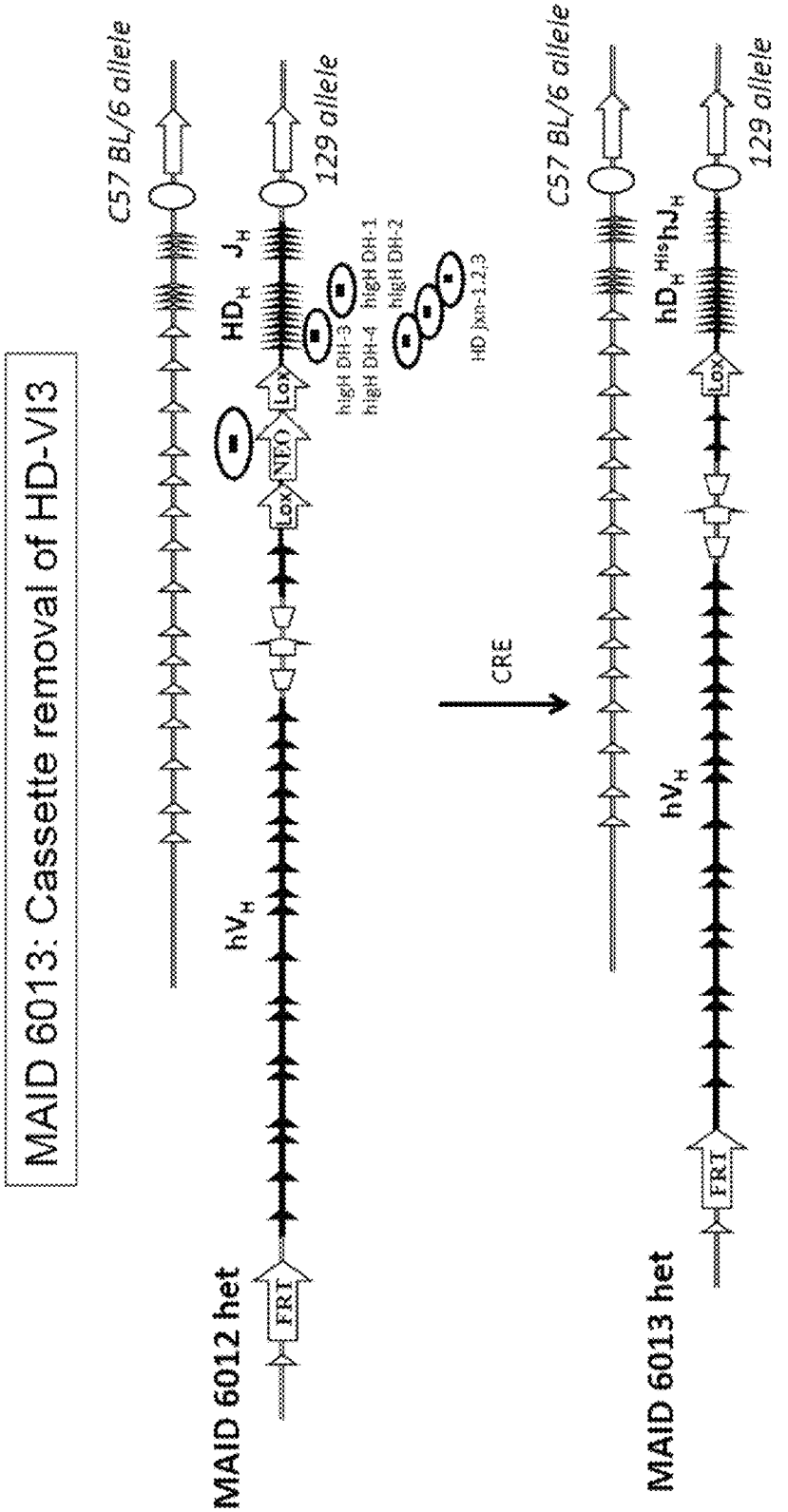

MAID 6013: Cassette removal of HD-VI3
C57 BL/6 allele
129 allele
MAID 6012 het
FRT
hV$_H$
HD$_H$
J$_H$
Lox
NEO
Lox
higH DH-3
higH DH-4
higH DH-1
higH DH-2
HD jxn-1,2,3
CRE
C57 BL/6 allele
129 allele
MAID 6013 het
hV$_H$
hD$_H$^{His}hJ$_H$
Lox
FRT

FIG. 9

Human D Gene Segment		Direct 5'-3' Orientation	SEQ ID NO.	Inverted Orientation	SEQ ID NO.
IGHD1-1	X97051, IGHD1-1*01	ggtacaactggaacgac	155	gtcgttccagtgtacc	206
		G T T G T	88	V V P V V	207
		V Q L E R	8	S F Q L Y	208
		Y N W N D	45	R S S C T	209
IGHD1-7	X13972, IGHD1-7*01	ggtataactggaactac	156	gtagttccagtatacc	210
		G I T G T	89	V V P V I	211
		V * L E L	-	* F Q L Y	212
		Y N W N Y	47	S S S Y T	213
IGHD 1-20	X13972, IGHD1-14*01	ggtataaccggaaccac	157	gtggttccggttatacc	214
		G I T G T	89	V V P V I	211
		V * P E P	-	W F R L Y	215
		Y N R N H	158	G S G Y T	216
IGHD 1-20	X97501, IGHD1-20*01	ggtataactggaacgac	159	gtcgttccagttatacc	217
		G I T G T	89	V V P V I	211
		V * L E R	-	S F Q L Y	208
		Y N W N D	45	R S S Y T	218
IGHD1-26	X97501, IGHD1-26*01	ggtatagtgggagctactac	160	gtagtagctcccactatacc	219
		G I V G A T	90	V V A P T I	220
		V * W E L L	12	* * L P L Y	221
		Y S G S Y Y	49	S S S H Y T	222
IGHD2-2	J00232, IGHD2-2*01	aggatattgtagtaccagctgctatgcc	161	ggcatagcagctgctactacaatatcct	223
		R I L * * Y Q L L C	162	G I A A G T T T I S	224
		G Y C S S T S C Y A	163	A * Q L V L L Q Y P	225
		D I V V P A A M	164	H S S W Y Y Y N I	226

FIG. 10A

Human D Gene Segment		Direct 5'- 3' Orientation	SEQ ID NO.	Inverted Orientation	SEQ ID NO.
IGHD2-8	X97051, IGHD2-2*02	aggatattgtagtagtaccagctgctatacc R I L * Y Q L L Y G Y C S S T S C Y T D I V V V P A A I	165 14 51 92	ggtatagcagctggtactactacaaatatcct G I A A G T T T I S V * Q L V L L Q Y P Y S S W Y Y Y Y N I	227 224 225 226
	M35648, IGHD2-2*03	tggatattgtagtagtaccagctgctatgcc W I L * Y Q L L C G Y C S S T S C Y A D I V V V P A A M	166 167 168 169	ggcatagcagctggtactactacaaatatcca G I A A G T T T I S A * Q L V L L Q Y P H S S W Y Y Y Y N I	228 224 225 226
	X13972, IGHD2-8*01	aggatattgtactaatggtggtatgctatacc R I L Y * W C M L Y G Y C T N G V C Y T D I V L M V Y A I	170 16, 17 53 94	ggtatagcatacaccaattagtacaatatcct G I A Y T I S T I S V * H T P L V Q Y P Y S I H H * Y N I	229 230 231 232
	J00233, IGHD2-8*02	aagatattgtactggtggtgtatgctatacc R I L Y W W C M L Y G Y C T G G V C Y T D I V L V V Y A I	171 172 173 174	ggtatagcatacaccaccagtacaatatctt G I A Y T T S T I S V * H T P P V Q Y L Y S I H H Q Y N I	233 234 235 236
IGHD2-15	J00234, IGHD2-15*01	aggatattgtagtggtggtagctgctactcc R I L * W W * L L L G Y C S G G S C Y S D I V V V A A T	175 - 55 95	ggagtagcagctaccaccactacaaatatcct G V A A T T T T I S E * Q L P P L Q Y P S S S Y H H Y N I	237 238 239 240
IGHD2-21	J00235, IGHD2-21*01	agcatattgtggtggtgattgctatcc S I L W W * L L F A Y C G G D C Y S H I V V V I A I	176 19 57 177	ggaaatagcaatcaccaccacaatatgct G I A I T T T T I C E * Q S P P Q Y A N S N H H H N M	241 242 243 244
	X97051, IGHD2-21*02	agcatattgtggtggactgctatttcc S I L W W * L L F A Y C G G D C Y S H I V V V T A I	178 19 57 97	ggaatagcagtcaccaccacaatatgct G I A V T T T T I C E * Q S P P Q Y A N S S H H H N M	245 246 247 248

FIG. 10B

Human D Gene Segment		Direct 5'-3' Orientation	SEQ ID NO.	Inverted Orientation	SEQ ID NO.
IGHD3-3	X13972, IGHD3-3*01	gtattacgatttttggagtggttattatacc V L R F L E W L L Y Y Y D F W S G Y Y T I T I F G V V I I	179 21 59 98	ggtataataaccactccaaaaatcgtaatac G I I T T P K I V I V * * P L Q K S * Y Y N N H S K N R N	249 250 251 252
	X93618, IGHD3-3*02	gtattagcatttttggagtggttattatacc V L A F L E W L L Y Y * H F W S G Y Y T I S I F G V V I I	180 181 182 183	ggtataataaccactccaaaaatgctaatac G I I T T P K M L I V * * P L Q K C * Y Y N N H S K N A N	253 254 255 256
IGHD3-9	X13972, IGHD3-9*01	gtattacgatattttgaccggttattaaac V L R Y F D W L L * Y Y D I L T G Y Y N I T I F * L V I I	184 23 61 99, 100	gttataataaccagtcaaaatatcgtaatac V I I T S Q N I V I L * * P V K I S * Y Y N N Q S K Y R N	257 258 259 260
IGHD3-10	X13972, IGHD3-10*01	gtattactatggttcgggggagttattataaac V L L W F G E L L * Y Y Y G S G S Y Y N I T M V R G V I I	185 25 63 101	gttataataactccccgaaccatagtaatac V I I T P R T I V I L * * L P E P * * Y Y N N S P N H S N	261 262 263 264
	X93615, IGHD3-10*02	gtattactatgttcgggggagttattataac V L L C S G S Y Y N Y Y Y V R G V I I I T M F G R L L *	186 187 188 189	gttataataactccccgaaccatagtaatac V I I T P R T * * Y L * * L P E H S N Y N N S P N I V I	265 266 267 268
IGHD3-16	X93614, IGHD3-16*01	gtattatgattacgttttgggggagttatgcttatacc V L * L R L G E L C L Y Y Y D Y V W G S Y A Y T I M I T F G G V M L I	190 191 192 193	ggtataagcataactcccccaaacgtaatcataatac G I S I T P P N V I I I V * A * L P Q T * S * Y Y K H N S P K R N H N	269 270 271 272
IGHD3-22	X93616, IGHD3-22*01	gtattactatgatagtggttattactac V L L * * W L L L Y Y Y D S G Y Y Y I T M I V V V I T	194 29 67 103	gtagtaataaccactatcatagtaatac V V I T T T I I V I * * * P L L S * * Y S N N H Y Y H S N	273 274 275 276

FIG. 10C

Human D Gene Segment		Direct 5'- 3' Orientation	SEQ ID NO.	Inverted Orientation	SEQ ID NO.
IGHD4-4	X13972, IGHD4-4*01	tgactacagtaactac * L Q * L D Y S N Y T T V T	195 - 69 105	gtagttactgtagtca V V T V V * L L * S S Y C S	277 278 - 279
IGHD4-11	X13972, IGHD4-11*01	tgactacagtaactac * L Q * L D Y S N Y T T V T	195 - 69 105	gtagttactgtagtca V V T V V * L L * S S Y C S	277 278 - 279
IGHD4-17	X97051, IGHD4-17*01	tgactacggtgactac * L R * L D Y G D Y T T V T	196 - 71 105	gtagtaccgtagtca V V T V V * S P * S S H R S	280 278 - 281
IGHD4-23	X97051, IGHD4-23*01	tgactacggtggtaactcc * L R W * L D Y G G N S T T V V T	197 - 73 106	ggagttaccaccgtagtca G V T T V V E L P P * S S Y H R S	282 283 284 285
IGHD5-5	X13972, IGHD5-5*01	gtggatacagctatggttac V D T A M V W I Q L W L G Y S Y G Y	198 107 35 75	gtaaccatagctgtatccac V T I A V S * P * L Y P N H S C I H	286 287 - 288
IGHD5-12	X13972, IGHD5-12*01	gtggatatagtggctacgattac V D I V A T I W I * W L R L G Y S G Y D Y	199 108 37 77	gtaatcgtagccactatatccac V I V A T I S * S * P L Y P N R S H Y I H	289 290 291 292
IGHD5-18	X97051, IGHD5-18*01	gtggatacagctatggttac V D T A M V W I Q L W L G Y S Y G Y	198 107 35 75	gtaaccatagctgtatccac V T I A V S * P * L Y P N H S C I H	286 287 - 288

FIG. 10D

Human D Gene Segment		Direct 5'- 3' Orientation	SEQ ID NO.	Inverted Orientation	SEQ ID NO.
IGHD5-24	X97051, IGHD5-24*01	gtagagatggctacaattac V E M A T I * R W L Q L R D G Y N Y	200 110 39 79	gtaattgtagccatctctac V I V A I S * L * P S L N C S H L Y	293 294 - 295
IGHD6-6	X13972, IGHD6-6*01	gagtatagcagctgctcc E Y S S S S S I A A R V * Q L V	201 81 112 -	ggacgagctgctatactc G R A A I L D E L L Y T S C Y T	296 297 298 299
IGHD6-13	X13972, IGHD6-13*01	gggtatagcagcagctggtac G Y S S S W Y G I A A A G V * Q Q L V	202 83 114 41	gtaccagctgctgctataccc V P A A A I P Y Q L L L Y T S C C Y T	300 301 302 303
IGHD6-19	X97051, IGHD6-19*01	gggtatagcagtggctggtac G Y S S G W Y G I A V A G V * Q W L V	203 85 116 44	gtaccagccactgctataccc V P A T A I P Y Q P L L Y T S H C Y T	304 305 306 307
IGHD6-25	X97051, IGHD6-25*01	gggtatagcagcggctac G Y S S G Y G I A A A V * Q R L	204 87 116 -	gtagccgctgctataccc V A A A I P * P L L Y S R C Y T	308 309 310 311
IGHD7-27	J00256, IGHD7-27*01	ctaactgggga L T G * L G N W G	205 - - -	tccccagttag S P V P Q L P S *	312 - - -

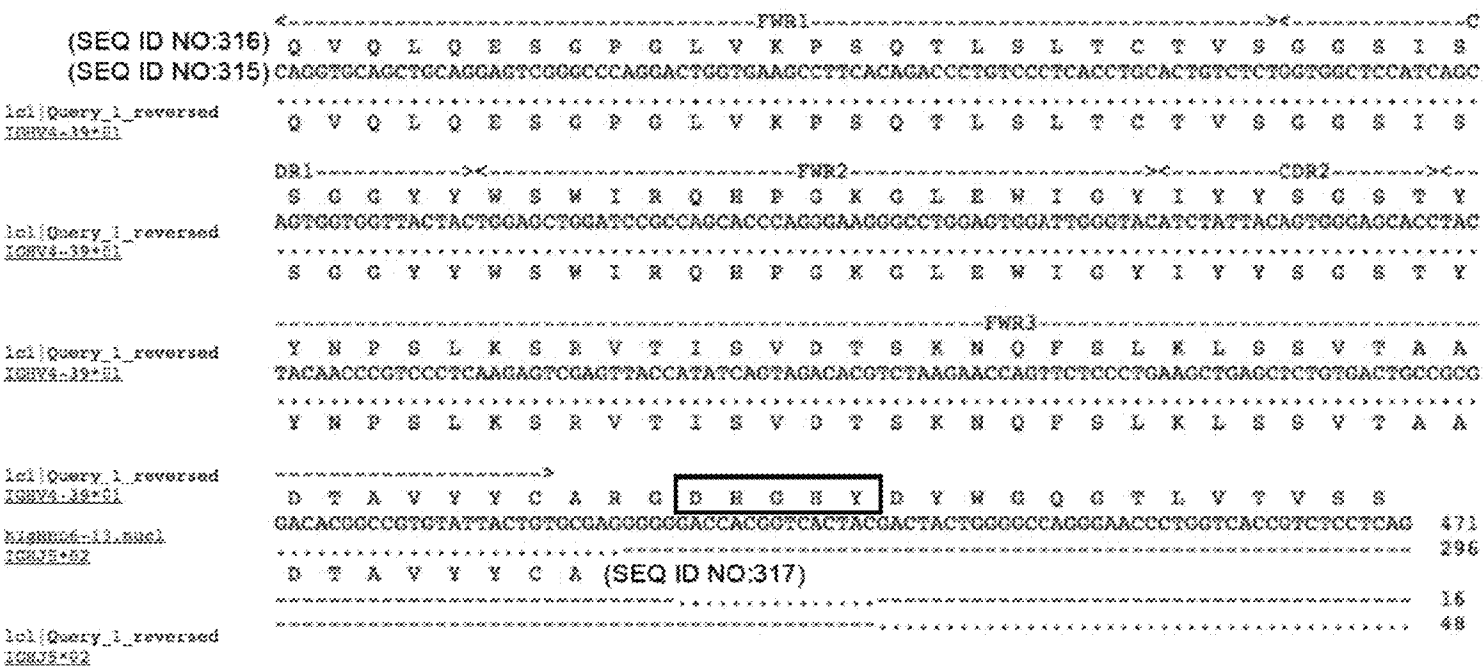
FIG. 10E

Antibody Sequence 1

D gene segment	Hydraphilic	SEQ ID NO.
D6-19	GYSSGWY	85
HD6-19	GHSHGWY	86

FIG. 11

Antibody Sequence 2



D gene segment	Hydrophilic	SEQ ID NO.
D4-17	DYGDY	71
HD4-17	DHGHY	72

FIG. 12

Antibody Sequence 3

D gene segment	Hydrophilic	SEQ ID NO.
D6-13	GYSSSWY	83
HD6-13	GHSHSWY	84

FIG. 13

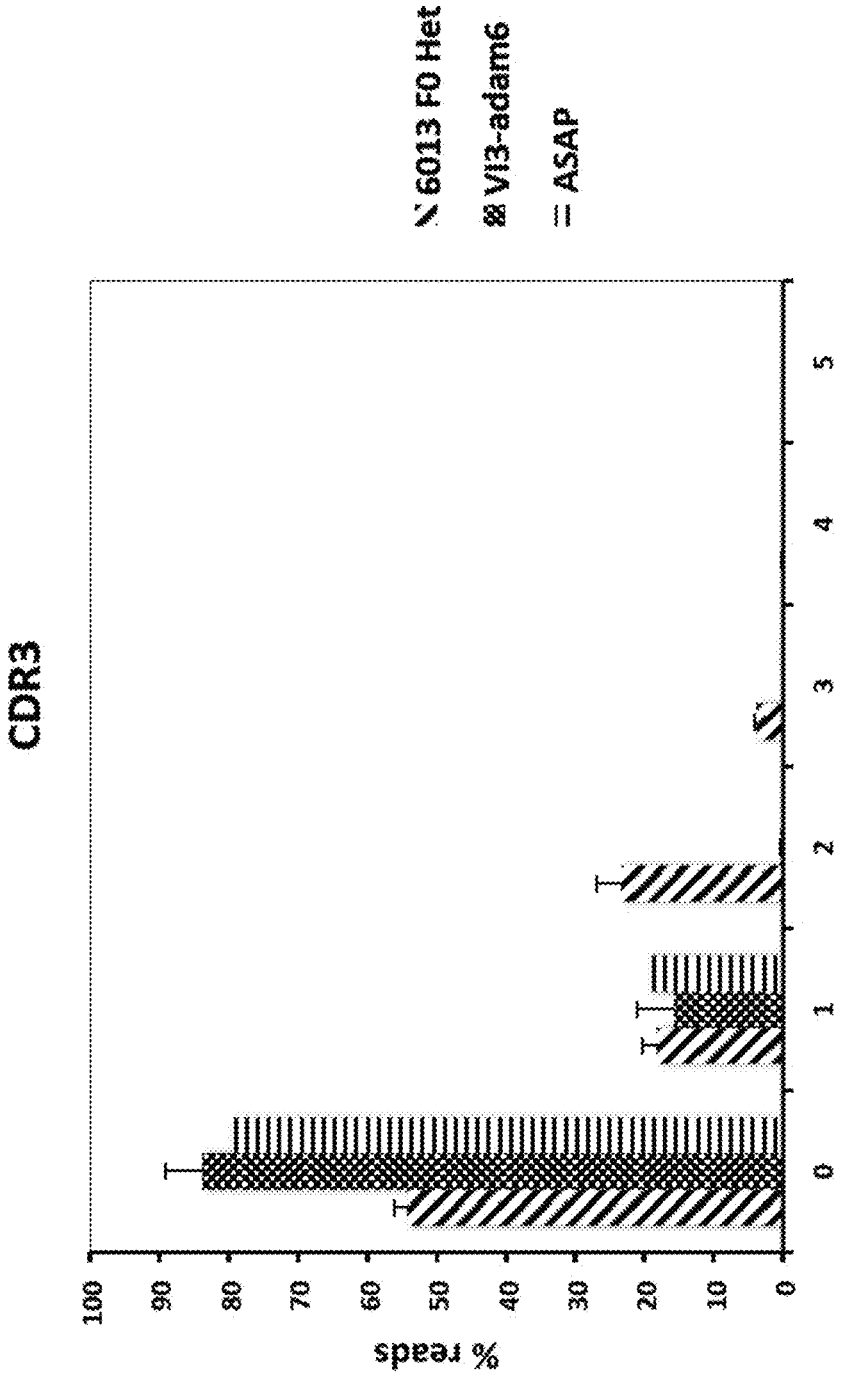

CDR3
6013 F0 Het
VI3-adam6
ASAP
% reads
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
His

FIG. 14

MICE THAT PRODUCE ANTIGEN-BINDING PROTEINS WITH PH-DEPENDENT BINDING CHARACTERISTICS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 61/611,950, filed 16 Mar. 2012, U.S. Provisional Application No. 61/613,352, filed Mar. 20, 2012, and U.S. Provisional Application No. 61/736,930, filed 13 Dec. 2012, the entire contents of each of the applications are incorporated herein by reference.

FIELD OF THE INVENTION

Genetically modified immunoglobulin loci of non-human animals comprising an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged human heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one non-histidine codon with a histidine codon. Non-human animals, including rodents, e.g., mice and rats, comprising in their germline an unrearranged human immunoglobulin heavy chain variable region nucleotide sequence, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one non-histidine codon with a histidine codon. Genetically engineered non-human animals capable of expressing an antigen-binding protein that is characterized by pH-dependent antigen binding, improved recyclability, and/or enhanced serum half-life.

BACKGROUND OF THE INVENTION

Drugs administered into the body, including therapeutic monoclonal antibodies, can be affected via various elimination mechanisms, including glomerular filtration (e.g., into urine), secretion (e.g., into the bile), and catabolism by cells. While small molecules are cleared from the body via renal filtration, the majority of secreted antibodies (e.g., IgG, which are too big to be filtered through glomeruli) are primarily removed from the body via cell-mediated catabolism, e.g., fluid-phase endocytosis (phagocytosis) or receptor-mediated endocytosis. For example, soluble molecules with several repeated epitopes are bound by a plurality of circulating antibodies, and the resulting large antigen-antibody complexes are phagocytosed rapidly into cells for degradation. On the other hand, cell surface target receptors, which are bound by antibodies (i.e., receptor-antibody complexes), undergo target-mediated endocytosis in a dose-dependent manner, which leads to formation of endosomes destined for lysosomal degradation inside cells. In some cases, the endocytosed receptor-antibody complexes bind neonatal Fc receptors (FcRn) inside the endosomes in a pH-dependent manner and are routed back to the cell surface for release into plasma or interstitial fluids upon exposure to a neutral extracellular pH (e.g., pH 7.0-7.4).

There is a need in the art for systems, e.g., non-human animals, cells, and genomic loci that generate antigen-binding proteins with titratable residues, e.g., genetically modified loci that rearrange immunoglobulin gene segments to generate heavy chain variable domains that respond to changes in pH, e.g., that donate or accept protons and, e.g., whose binding characteristics differ according to protonation state.

There is also a need in the art for methods and compositions that can further increase recycling efficiency of endocytosed antigen-binding proteins by promoting dissociation of antigen-binding proteins from receptor-antigen-binding protein complexes or by increasing the affinity of antigen-binding proteins toward FcRn in an acidic endosomal compartment without compromising the specificity and affinity of the antigen-binding protein toward an antigen of interest.

SUMMARY OF THE INVENTION

Genetically modified immunoglobulin heavy chain loci in the germline genome of non-human animals are provided, wherein the immunoglobulin heavy chain loci comprise a genetically modified unrearranged heavy chain variable region nucleotide sequence (e.g., one or more genetically modified human V_H, D, and/or J_H gene segment), wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of at least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon. In various embodiments, the genetically modified unrearranged heavy chain variable region nucleotide sequence comprises at least one histidine codon in at least one reading frame that encodes an immunoglobulin heavy chain variable domain. In various embodiments, the unrearranged heavy chain variable region nucleotide sequence comprising the at least one histidine codon is operably linked to a human or non-human heavy chain constant region nucleotide sequence (e.g., a heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgA, IgE, and IgG).

Non-human animals (mammals, e.g., rodents such as mice, rats, or hamsters) are provided that are genetically engineered to contain immunoglobulin heavy chain genomic loci in their germline genome, wherein the genomic loci comprise an unrearranged heavy chain variable region nucleotide sequence (e.g., one or more genetically modified human V_H, D, and/or J_H gene segments), wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of at least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon. In various embodiments, the genome of the non-human animals comprises a modification (i) that deletes or renders nonfunctional all, or substantially all, endogenous immunoglobulin V_H, D, and/or J_H gene segments (e.g., via insertion of a nucleotide sequence, e.g., an exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement or inversion of endogenous V_H, D, and/or J_H gene segments); and (ii) that introduces an unrearranged human heavy chain variable region nucleotide sequence (e.g., genetically modified human V_H, D, or J_H gene segments), wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of at least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon. In various embodiments, the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous locus (i.e., where the unrearranged heavy chain variable region nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin heavy chain locus in its genome), or within its endogenous locus (e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome). In various embodiments, the immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence

(e.g., a heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgA, IgE, and IgG).

Genetically modified non-human animals are provided that are capable of expressing a genetically modified immunoglobulin heavy variable domain comprising one or more histidines, wherein the one or more histidines are not encoded by a germline gene segment of a corresponding wild-type non-human animal.

Genetically modified non-human animals are provided that comprise a B cell population that is characterized by rearranged immunoglobulin heavy chain variable genes that encode an immunoglobulin heavy chain variable domain with one or more histidines that are not encoded by a germline gene segment of a corresponding wild-type non-human animal.

Methods and compositions are provided for making non-human animals that comprise a genetically modified immunoglobulin heavy chain variable locus comprising an unrearranged human heavy chain variable region nucleotide sequence containing one or more histidine codons in at least one reading frame that encodes a heavy chain variable domain.

Methods and compositions are provided for non-human animals that make antigen-binding proteins that exhibit a pH-dependent binding of an antigen. Methods and compositions are provided for making non-human animals that have B cell populations, or antibody populations, that are enriched (as compared with corresponding wild-type animals) with antigen-binding proteins that are pH-dependent, e.g., in particular, heavy chain variable domains, and/or antigen-binding fragments thereof.

In one aspect, a genetically modified immunoglobulin locus in a germline genome of a non-human animal is provided comprising an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon.

In one embodiment, the non-human animal is a mammal, including a rodent, e.g., a mouse, a rat, or a hamster.

In one embodiment, the added or substituted histidine codon is present in an immunoglobulin heavy chain gene segment selected from a human V_H gene segment, a human D gene segment, a human J_H gene segment, and a combination thereof. In one embodiment, the immunoglobulin heavy chain gene segment is selected from a human germline V_H gene segment, a human germline D gene segment, a human germline J_H gene segment, and a combination thereof.

In one embodiment, the human V gene segment (V_H) is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, and a combination thereof.

In one embodiment, the human D gene segment is selected from the group consisting of D1-1, D1-7, D1-14, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-12, D5-5, D5-18, D5-24, D6-6, D6-13, D6-19, D6-25, D7-27, and a combination thereof.

In one embodiment, the human J gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, and a combination thereof.

In one embodiment, the added or substituted histidine codon is present in the unrearranged heavy chain variable region nucleotide sequence that encodes an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, or a combination thereof.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprises 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, or 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of histidine codons.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (i.e., C_H1-hinge-C_H2-C_H3).

In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., L/R/S/P/Q or K) and/or 434 (e.g., H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (e.g., 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (e.g., M428L) and 434S (e.g., N434S) modification; a 428L, 259I (e.g., V259I), and 308F (e.g., V308F) modification; a 433K (e.g., H433K) and a 434 (e.g., 434Y) modification; a 252, 254, and 256 (e.g., 252Y, 254T, and 256E) modification; a 250Q and 428L modification (e.g., T250Q and M428L); and a 307 and/or 308 modification (e.g., 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid

sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence (e.g., an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, e.g., about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, e.g., at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

In one embodiment, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders non-functional all, or substantially all, endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising one or more human V_H, D, and/or J_H gene segments having one or more histidine codons, wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus (e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the genetically modified immunoglobulin locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse λ locus.

In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (e.g., at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, e.g., antibodies, comprising a heavy chain variable domain with one or more histidine residues. The antigen-binding proteins as described herein, when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification as described herein.

In one aspect, a genetically modified immunoglobulin locus in a germline genome of a non-human animal is provided comprising an unrearranged human heavy chain variable region nucleotide sequence, wherein the human unrearranged heavy chain variable region nucleotide sequence comprises a substitution of at least one endogenous non-histidine codon with a histidine codon.

In one embodiment, the non-human animal is a mammal, including a rodent, e.g., a mouse, a rat, or a hamster.

In one embodiment, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more,

51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of the endogenous non-histidine codons are replaced with histidine codons.

In one embodiment, the endogenous non-histone codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes an immunoglobulin variable domain selected from an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, a combination thereof.

In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a complementary determining region (CDR) selected from a CDR1, a CDR2, a CDR3, and a combination thereof.

In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a frame region (FR) selected from FR1, FR2, FR3, FR4, and a combination thereof.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human V_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human V_H gene segment has been replaced with a histidine codon.

In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human V_H gene segment with a histidine codon, wherein the human V_H gene segment is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, and a combination thereof.

In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human J_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human J_H gene segment has been replaced with a histidine codon.

In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human J_H segment with a histidine codon, wherein the human J_H gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_HS, J_H6, and a combination thereof.

In one embodiment, the substituted histidine codon is present in a heavy chain variable region nucleotide sequence that encodes part of a CDR3. In one embodiment, the part of CDR3 comprises an amino acid sequence derived from a reading frame of a genetically modified human D gene segment comprising a modification that replaces at least one endogenous non-histidine codon in the reading frame with a histidine codon.

In one embodiment, the endogenous non-histidine codon that is substituted with a histidine codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

In one embodiment, the substituted histidine codon is present in at least one reading frame of the human D gene segment that is most frequently observed in VELOCIMMUNE® humanized immunoglobulin mice.

In one embodiment, the reading frame of the genetically modified human D gene segment that encodes part of CDR3 is selected from a hydrophobic frame, a stop frame, and a hydrophilic frame.

In one embodiment, the reading frame is a hydrophobic frame of a human D gene segment.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (GTTGT; SEQ ID NO: 88), D1-7 (GITGT; SEQ ID NO: 89), D1-20 (GITGT; SEQ ID NO: 89), and D1-26 (GIVGAT; SEQ ID NO: 90), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (DIVVVPAAI; SEQ ID NO: 92), D2-8 (DIVLMVYAI; SEQ ID NO: 94), D2-15 (DIVVVVAAT; SEQ ID NO: 95), and D2-21 (HIVVVTAI; SEQ ID NO: 97), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (ITIFGVVII; SEQ ID NO: 98), D3-9 (ITIF*LVII; SEQ ID NO: 99, SEQ ID NO:100), D3-10 (ITMVRGVII; SEQ ID NO:101), D3-16 (IMITFGGVIVI; SEQ ID NO:102), and D3-22 (ITMIVVVIT; SEQ ID NO:103), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (TTVT; SEQ ID NO: 105), D4-11 (TTVT; SEQ ID NO:105), D4-17 (TTVT; SEQ ID NO:105), D4-23 (TTVVT; SEQ ID NO: 106) and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (VDTAMV; SEQ ID NO: 107), D5-12 (VDIVATI; SEQ ID NO: 108), D5-18 (VDTAMV; SEQ ID NO:107), and D5-24 (VEMATI; SEQ ID NO:109), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (SIAAR; SEQ ID NO: 111), D6-13 (GIAAAG; SEQ ID NO: 113), and D6-19 (GIAVAG; SEQ ID NO:115), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame comprises a nucleotide sequence that encodes human D7-27 (LTG), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the reading frame is a stop reading frame of a human D gene segment.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (VQLER; SEQ ID NO:8), D1-7 (V*LEL), D1-20 (V*LER), D1-26 (V*WELL; SEQ ID NO: 12), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (RIL**YQLLY; SEQ ID NO:14), D2-8 (RILY*WCMLY; SEQ ID NO:16 and SEQ ID NO: 17), D2-15 (RIL*WW*LLL), and D2-21 (SILWW*LLF; SEQ ID NO:19), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (VLRFLEWLLY; SEQ ID NO:21), D3-9 (VLRYFDWLL*; SEQ ID NO:23), D3-10 (VLLWFGELL*; SEQ ID NO:25), D3-16 (VL*LRLGELSLY; SEQ ID NO:27), and D3-22 (VLL***WLLL; SEQ ID NO:29), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (*LQ*L), D4-11 (*LQ*L), D4-17 (*LR*L), and D4-23 (*LRW*L), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (WIQLWL; SEQ ID NO:35); D5-12 (Wl*WLRL; SEQ ID NO:37), D5-18 (WIQLWL; SEQ ID NO:35), and D5-24 (*RWLQL; SEQ ID NO:39), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (V*QLV), D6-13 (V*QQLV; SEQ ID NO:41), and D6-19 (V*QWLV; SEQ ID NO:43), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (*LG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon of the human D gene segment in the nucleotide sequence with a histidine codon.

In one embodiment, the reading frame is a hydrophilic frame of a human D gene segment.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (YNWND; SEQ ID NO: 45), D1-7 (YNWNY; SEQ ID NO: 47), D1-20 (YNWND; SEQ ID NO: 45), and D1-26 (YSGSYY; SEQ ID NO:49), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (GYCSSTSCYT; SEQ ID NO:51), D2-8 (GYCTNGVCYT; SEQ ID NO: 53), D2-15 (GYCSGGSCYS; SEQ ID NO:55), and D2-21 (AYCGGDCYS; SEQ ID NO:57), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (YYDFWSGYYT; SEQ ID NO:59), D3-9 (YYDILTGYYN; SEQ ID NO:61), D3-10 (YYYGSGSYYN; SEQ ID NO:63), D3-16 (YYDYVWGSYRYT; SEQ ID NO:65), and D3-22 (YYYDSSGYYY; SEQ ID NO:67), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (DYSNY; SEQ ID NO:69), D4-11 (DYSNY; SEQ ID NO:69), D4-17 (DYGDY; SEQ ID NO:71), and D4-23 (DYGGNS; SEQ ID NO:73), and the human D gene segment comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (GYSYGY; SEQ ID NO:75), D5-12 (GYSGYDY; SEQ ID NO:77), D5-18 (GYSYGY; SEQ ID NO:75), and D5-24 (RDGYNY; SEQ ID NO:79), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (EYSSSS; SEQ ID NO: 81), D6-13 (GYSSSWY; SEQ ID NO:83), and D6-19 (GYSSGWY; SEQ ID NO:85), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodi-

ment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 76, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (NWG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence a histidine codon.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, and a combination thereof.

In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (i.e., C_H1-hinge-C_H2-C_H3).

In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., L/R/S/P/Q or K) and/or 434 (e.g., H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (e.g., 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (e.g., M428L) and 434S (e.g., N434S) modification; a 428L, 259I (e.g., V259I), and 308F (e.g., V308F) modification; a 433K (e.g., H433K) and a 434 (e.g., 434Y) modification; a 252, 254, and 256 (e.g., 252Y, 254T, and 256E) modification; a 250Q and 428L modification (e.g., T250Q and M428L); and a 307 and/or 308 modification (e.g., 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon num-

bering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence, e.g., an exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, e.g., about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, e.g., at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

In one embodiment, the genetically modified locus comprises a modification that deletes or renders non-functional all or substantially all endogenous V_H, D, and J_H gene segments; and the genomic locus comprises a genetically modified, unrearranged human heavy chain variable region nucleotide sequence comprising a substitution of at least one endogenous non-histidine codon with a histidine codon in at least one reading frame. In one embodiment, the genetically modified, unrearranged immunoglobulin heavy chain variable gene sequence is present at an endogenous location (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

In one embodiment, the genetically modified locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

In one embodiment, the genetically modified locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment the mouse immunoglobulin light chain locus is a mouse λ locus.

In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (e.g., at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification described herein.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as

described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, e.g., antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins, which are produced by the genetically modified immunoglobulin locus described herein, when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

In one aspect, a genetically modified immunoglobulin locus of a non-human animal comprising a human V_H, D, and J_H gene segment is provided, wherein at least one human D gene segment has been inverted 5' to 3' with respect to a corresponding wild-type sequence, and wherein at least one reading frame of the inverted human D gene segment comprises one ore more histidine codon.

In one embodiment, the non-human animal is a mammal, including a rodent, e.g., a mouse, a rat, or a hamster

In one embodiment, the genetically modified immunoglobulin locus is present in a germline genome.

In one embodiment, the genetically modified immunoglobulin locus encodes an immunoglobulin heavy chain variable domain comprising one or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, or 34 or more of histidine residues.

In one embodiment, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty one, at least twenty two, at least twenty three, at least twenty four, or all or substantially all of functional human D gene segments have inverted orientation with respect to corresponding wild type sequences.

In one embodiment, all or substantially all of endogenous immunoglobulin V_H, D, J_H gene segments are deleted from the immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence, e.g., exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement or inversion of all, or substantially all, endogenous immunoglobulin V_H, D, J_H segments), and the genetically modified immunoglobulin locus comprises a human V_H, D, and J_H gene segments, wherein at least one human D gene segment is present in an inverted orientation with respect to a corresponding wild type sequence, and wherein at least one reading frame in the inverted human D gene segment comprises at least one histidine codon.

In one embodiment, the inverted human D gene segment is operably linked to a human V_H gene segment, and/or human J_H gene segment

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is selected from the group consisting of D1-1, D1-7, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-5, D5-12, D5-18, D5-24, D6-6, D6-13, D6-19, D7-27, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D1 gene segment selected from the group consisting of D1-1, D1-7, D1-20, D1-26, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative a corresponding wild type sequence is a D2 gene segment selected from the group consisting of D2-2, D2-8, D2-15, D2-21, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D3 gene segment selected from the group consisting of D3-3, D3-9, D3-10, D3-16, D3-22, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D4 gene segment selected from the group consisting of D4-4, D4-11, D4-17, D4-23, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D5 gene segment selected from the group consisting of D5-5, D5-12, D5-18, D5-24, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D6 gene segment selected from the group consisting of D6-6, D6-13, D6-19, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is D7-27.

In one embodiment, the reading frame of the human D gene segment is selected from a stop reading frame, a hydrophilic reading frame, and a hydrophobic reading frame, and at least one reading frame of the inverted human D gene segment comprises one or more histidine codon.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (i.e., C_H1-hinge-C_H2-C_H3).

In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., L/R/S/P/Q or K) and/or 434 (e.g., H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (e.g., 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (e.g., M428L) and 434S (e.g., N434S) modification; a 428L, 259I (e.g., V259I), and 308F (e.g., V308F) modification; a 433K (e.g., H433K) and a 434 (e.g., 434Y) modification; a 252, 254, and 256 (e.g., 252Y, 254T, and 256E) modification; a 250Q and 428L modification (e.g., T250Q and M428L); and a 307 and/or 308 modification (e.g., 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, incorporated by reference herein in its entirety).

In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, all, or substantially all, endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence (e.g., an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, e.g., about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, e.g., at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

In one embodiment, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders non-functional, all or substantially all, endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising at least one inverted human D gene segment as described herein wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the genetically modified immunoglobulin locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse immunoglobulin light chain locus is a mouse λ locus.

In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (e.g., at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein compris-

ing a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, e.g., antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

In one aspect, a non-human animal is provided comprising in its germline genome a genetically modified immunoglobulin locus comprising an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon.

In one embodiment, the non-human animal is a mammal, including a rodent, e.g., a mouse, a rat, or a hamster.

In one embodiment, the added or substituted histidine codon is present in an immunoglobulin heavy chain gene segment selected from a human V_H gene segment, a human D gene segment, a human J_H gene segment, and a combination thereof. In one embodiment, the immunoglobulin heavy chain gene segment is selected from a human germline V_H gene segment, a human germline D gene segment, a human germline J_H gene segment, and a combination thereof.

In one embodiment, the human V_H gene segment is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, and a combination thereof.

In one embodiment, the human D gene segment is selected from the group consisting of D1-1, D1-7, D1-14, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-12, D5-5, D5-18, D5-24, D6-6, D6-13, D6-19, D6-25, D7-27, and a combination thereof.

In one embodiment, the human J_H gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, and a combination thereof.

In one embodiment, the added or substituted histidine codon is present in the unrearranged heavy chain variable region nucleotide sequence encoding an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, or a combination thereof.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprises 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, or 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of histidine codons.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (i.e., C_H1-hinge-C_H2-C_H3).

In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., L/R/S/P/Q or K) and/or 434 (e.g., H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or

308 (e.g., 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (e.g., M428L) and 434S (e.g., N434S) modification; a 428L, 259I (e.g., V259I), and 308F (e.g., V308F) modification; a 433K (e.g., H433K) and a 434 (e.g., 434Y) modification; a 252, 254, and 256 (e.g., 252Y, 254T, and 256E) modification; a 250Q and 428L modification (e.g., T250Q and M428L); and a 307 and/or 308 modification (e.g., 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence (e.g., an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, e.g., about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, e.g., at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

In one embodiment, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders, all or substantially all, non-functional endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising one or more human V_H, D, and/or J_H gene segments having one or more histidine codons, wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (i.e., where the nucleotide sequence is located in a wild-type non-

human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the genetically modified immunoglobulin locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse λ locus.

In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

In one embodiment, the non-human animal is heterozygous for the genetically modified immunoglobulin heavy chain locus, and the non-human animal is capable of expressing a human immunoglobulin heavy chain variable domain comprising at least one histidine residue derived predominantly from the genetically modified immunoglobulin heavy chain locus as described herein.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (e.g., at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, e.g., antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

In one aspect, a non-human animal comprising a genetically modified immunoglobulin locus is provided, wherein the genetically modified immunoglobulin locus comprises an unrearranged human heavy chain variable region nucleotide sequence, and wherein the human unrearranged heavy chain variable region nucleotide sequence comprises a substitution of at least one endogenous non-histidine codon with a histidine codon.

In one embodiment, the non-human animal is a mammal, including a rodent, e.g., a mouse, a rat, or a hamster.

In one embodiment, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of the endogenous non-histidine codons are replaced with histidine codons.

In one embodiment, the endogenous non-histone codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes an immunoglobulin variable domain selected from an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, a combination thereof.

In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a complementary determining region (CDR) selected from a CDR1, a CDR2, a CDR3, and a combination thereof.

In one embodiment, the substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a frame region (FR) selected from FR1, FR2, FR3, FR4, and a combination thereof.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human V_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human V_H gene segment has been replaced with a histidine codon.

In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human V_H gene segment with a histidine codon, wherein the human V_H gene segment is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, and a combination thereof.

In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human J_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human J_H gene segment has been replaced with a histidine codon.

In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human J_H segment with a histidine codon, wherein the human J_H gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_HS, J_H6, and a combination thereof.

In one embodiment, the substituted histidine codon is present in a heavy chain variable region nucleotide sequence that encodes part of a CDR3. In one embodiment, the part of CDR3 comprises an amino acid sequence derived from a reading frame of a genetically modified human D gene segment comprising a modification that replaces at least one endogenous non-histidine codon in the reading frame with a histidine codon.

In one embodiment, the endogenous non-histidine codon that is substituted with a histidine codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

In one embodiment, the substituted histidine codon is present in at least one reading frame of the human D gene segment that is most frequently observed in VELOCIMMUNE® humanized immunoglobulin mice.

In one embodiment, the reading frame of the genetically modified human D gene segment that encodes part of CDR3 is selected from a hydrophobic frame, a stop frame, and a hydrophilic frame.

In one embodiment, the reading frame is a hydrophobic frame of a human D gene segment.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (GTTGT; SEQ ID NO: 88), D1-7 (GITGT; SEQ ID NO: 89), D1-20 (GITGT; SEQ ID NO: 89), and D1-26 (GIVGAT; SEQ ID NO:90), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (DIVVVPAAI; SEQ ID NO:92), D2-8 (DIVLMVYAI; SEQ ID NO: 94), D2-15 (DIVVVVAAT; SEQ ID NO:95), and D2-21 (HIVVVTAI; SEQ ID NO: 97), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (ITIFGVVII; SEQ ID NO:98), D3-9 (ITIF*LVII; SEQ ID NO:99, SEQ ID NO:100), D3-10 (ITMVRGVII; SEQ ID NO:101), D3-16 (IMITFGGVIVI; SEQ ID NO:102), and D3-22 (ITMIVVVIT; SEQ ID NO:103), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (TTVT; SEQ ID NO:105), D4-11 (TTVT; SEQ ID NO:105), D4-17 (TTVT; SEQ ID NO:105), D4-23 (TTVVT; SEQ ID NO: 106) and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes

the amino acid sequence selected from the group consisting of D5-5 (VDTAMV; SEQ ID NO: 107), D5-12 (VDIVATI; SEQ ID NO:108), D5-18 (VDTAMV; SEQ ID NO:107), and D5-24 (VEMATI; SEQ ID NO:109), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (SIAAR; SEQ ID NO:111), D6-13 (GIAAAG; SEQ ID NO:113), and D6-19 (GIAVAG; SEQ ID NO:115), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame comprises a nucleotide sequence that encodes human D7-27 (LTG), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the reading frame is a stop reading frame of a human D gene segment.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (VQLER; SEQ ID NO:8), D1-7 (V*LEL), D1-20 (V*LER), D1-26 (V*WELL; SEQ ID NO:12), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (RIL**YQLLY; SEQ ID NO:14), D2-8 (RILY*WCMLY; SEQ ID NO:16 and SEQ ID NO: 17), D2-15 (RIL*WW*LLL), and D2-21 (SILWW*LLF; SEQ ID NO:19), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (VLRFLEWLLY; SEQ ID NO:21), D3-9 (VLRYFDWLL*; SEQ ID NO:23), D3-10 (VLLWFGELL*; SEQ ID NO:25), D3-16 (VL*LRLGELSLY; SEQ ID NO:27), and D3-22 (VLL***WLLL; SEQ ID NO:29), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (*LQ*L), D4-11 (*LQ*L), D4-17 (*LR*L), and D4-23 (*LRW*L), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (WIQLWL; SEQ ID NO:35); D5-12 (WI*WLRL; SEQ ID NO:37), D5-18 (WIQLWL; SEQ ID NO:35), and D5-24 (*RWLQL; SEQ ID NO:39), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (V*QLV), D6-13 (V*QQLV; SEQ ID NO:41), and D6-19 (V*QWLV; SEQ ID NO:43), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (*LG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon of the human D gene segment in the nucleotide sequence with a histidine codon.

In one embodiment, the reading frame is a hydrophilic frame of a human D gene segment.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (YNWND; SEQ ID NO: 45), D1-7 (YNWNY; SEQ ID NO: 47), D1-20 (YNWND; SEQ ID NO: 45), and D1-26 (YSGSYY; SEQ ID NO:49), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (GYCSSTSCYT; SEQ ID NO:51), D2-8 (GYCTNGVCYT; SEQ ID NO: 53), D2-15 (GYCSGGSCYS; SEQ ID NO:55), and D2-21 (AYCGGDCYS; SEQ ID NO:57), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (YYDFWSGYYT; SEQ ID NO:59), D3-9 (YYDILTGYYN; SEQ ID NO:61), D3-10 (YYYGSGSYYN; SEQ ID NO:63), D3-16 (YYDYVWGSYRYT; SEQ ID NO:65), and D3-22 (YYYDSSGYYY; SEQ ID NO:67), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (DYSNY; SEQ ID NO:69), D4-11 (DYSNY; SEQ ID NO:69), D4-17 (DYGDY; SEQ ID NO:71), and D4-23 (DYGGNS; SEQ ID NO:73), and the human D gene segment comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (GYSYGY; SEQ ID NO:75), D5-12 (GYSGYDY; SEQ ID NO:77), D5-18 (GYSYGY; SEQ ID NO:75), and D5-24 (RDGYNY; SEQ ID NO:79), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (EYSSSS; SEQ ID NO: 81), D6-13 (GYSSSWY; SEQ ID NO:83), and D6-19 (GYSSGWY; SEQ ID NO:85), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 76, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (NWG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence a histidine codon.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, and a combination thereof.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (i.e., C_H1-hinge-C_H2-C_H3).

In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., L/R/S/P/Q or K) and/or 434 (e.g., H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (e.g., 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (e.g., M428L) and 434S (e.g., N434S) modification; a 428L, 259I (e.g., V259I), and 308F (e.g., V308F) modification; a 433K (e.g., H433K) and a 434 (e.g., 434Y) modification; a 252, 254, and 256 (e.g., 252Y, 254T, and 256E) modification; a 250Q and 428L modification (e.g., T250Q and M428L); and a 307 and/or 308 modification (e.g., 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, all, or substantially all, endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence (e.g., an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, e.g., about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, e.g., at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

In one embodiment, the genetically modified locus comprises a modification that deletes or renders non-functional all or substantially all endogenous V_H, D, and J_H gene segments; and the genomic locus comprises the genetically modified, unrearranged human heavy chain variable region nucleotide sequence comprising a substitution of at least one endogenous non-histidine codon with a histidine codon in at least one reading frame. In one embodiment, the genetically modified, unrearranged immunoglobulin heavy chain variable gene sequence is present at an endogenous location (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

In one embodiment, the genetically modified locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

In one embodiment, the genetically modified locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse

immunoglobulin light chain locus is a mouse κ locus. In one embodiment the mouse immunoglobulin light chain locus is a mouse λ locus.

In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (e.g., at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

In one embodiment, the genetically modified immunoglobulin locus as described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, e.g., antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein, when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

In one embodiment, the non-human animal is heterozygous for the genetically modified immunoglobulin heavy chain locus, and the non-human animal is capable of expressing the human immunoglobulin heavy chain variable domain comprising at least one histidine residue derived predominantly from the genetically modified immunoglobulin heavy chain locus as described herein.

In one aspect, a non-human animal comprising a genetically modified immunoglobulin locus comprising a human V_H, D, and J_H gene segment is provided, wherein at least one of the human D gene segment has been inverted 5' to 3' with respect to a corresponding wild-type sequence, and wherein at least one reading frame of the inverted human D gene segment comprises a histidine codon.

In one embodiment, the non-human animal is a mammal, including a rodent, e.g., a mouse, a rat, or a hamster

In one embodiment, the genetically modified immunoglobulin locus is present in a germline genome.

In one embodiment, wherein the reading frame of the inverted human D gene segment comprises one or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, or 34 or more of histidine codons.

In one embodiment, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty one, at least twenty two, at least twenty three, at least twenty four, or all or substantially all of functional human D gene segments have inverted orientation with respect to corresponding wild type sequences.

In one embodiment, all or substantially all of endogenous immunoglobulin V_H, D, J_H gene segments are deleted from the immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence, e.g., exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement or inversion of all, or substantially all, endogenous immunoglobulin V_H, D, J_H segments), and the genetically modified immunoglobulin locus comprises a human V_H, D, and J_H gene segments, wherein at least one of the human D gene segment is present in an inverted orientation with respect to corresponding wild type sequences, and wherein at least one reading frame of the inverted human D gene segment comprises at least one histidine codon.

In one embodiment, the inverted human D gene segment is operably linked to a human V_H gene segment, and/or human J_H gene segment

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is selected from the group consisting of D1-1, D1-7, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-5, D5-12, D5-18, D5-24, D6-6, D6-13, D6-19, D7-27, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D1 gene segment selected from the group consisting of D1-1, D1-7, D1-20, D1-26, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequences is a D2 gene segment selected from the group consisting of D2-2, D2-8, D2-15, D2-21, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D3 gene segment selected from the group consisting of D3-3, D3-9, D3-10, D3-16, D3-22, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D4 gene segment selected from the group consisting of D4-4, D4-11, D4-17, D4-23, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D5 gene segment selected from the group consisting of D5-5, D5-12, D5-18, D5-24, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D6 gene segment selected from the group consisting of D6-6, D6-13, D6-19, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is D_7-27.

In one embodiment, the reading frame of the human D gene segment is selected from a stop reading frame, a hydrophilic reading frame, a hydrophobic reading frame, and a combination thereof, wherein at least one reading frame of the inverted human D gene segment comprises a histidine codon.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (i.e., C_H1-hinge-C_H2-C_H3).

In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., L/R/S/P/Q or K) and/or 434 (e.g., H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (e.g., 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (e.g., M428L) and 434S (e.g., N434S) modification; a 428L, 259I (e.g., V259I), and 308F (e.g., V308F) modification; a 433K (e.g., H433K) and a 434 (e.g., 434Y) modification; a 252, 254, and 256 (e.g., 252Y, 254T, and 256E) modification; a 250Q and 428L modification (e.g., T250Q and M428L); and a 307 and/or 308 modification (e.g., 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, incorporated by reference herein in its entirety).

In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence (e.g., an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, e.g., about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, e.g., at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

In one embodiment, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders, all or substantially all, non-functional endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising at least one inverted human D gene segment as described herein wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the genetically modified immunoglobulin locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse immunoglobulin light chain locus is a mouse λ locus.

In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglo-

bulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

In one embodiment, the non-human animal is heterozygous for the genetically modified immunoglobulin heavy chain locus, and the non-human animal is capable of expressing the human immunoglobulin heavy chain variable domain comprising at least one histidine residue derived predominantly from the genetically modified immunoglobulin heavy chain locus as described herein.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (e.g., at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, e.g., antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

In one aspect, a non-human animal that is capable of expressing an antigen-binding protein with enhanced pH-dependent recyclability and/or enhanced serum half-life are provided, wherein the non-human animal comprises in its germline genome an unrearranged human immunoglobulin heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon as described herein.

In one embodiment, the antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (e.g., at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, the antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, e.g., antibodies, comprising a heavy chain variable domain comprising one or

more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

In one aspect, a targeting construct is provided, comprising 5' and 3' targeting arms homologous to a genomic D region or genomic V and J region of a non-human animal, wherein at least one V_H, D, or J_H gene segment comprises any of the modifications as described herein, e.g., an addition of at least one histidine codon, a substitution of at least one endogenous non-histidine codon into a histidine codon, and/or inversion of at least one functional D gene segment with respect to a corresponding wild type sequence.

In one aspect, a hybridoma or quadroma is provided that is derived from a cell of any of the non-human animal as described herein. In one embodiment, the non-human animal is a rodent, e.g., a mouse, a rat, or a hamster.

In one aspect, pluripotent, induced pluripotent, or totipotent stem cells derived form a non-human animal comprising the various genomic modifications of the described invention are provided. In a specific embodiment, the pluripotent, induced pluripotent, or totipotent stem cells are mouse or rat embryonic stem (ES) cells. In one embodiment, the pluripotent, induced pluripotent, or totipotent stem cells have an XX karyotype or an XY karyotype. In one embodiment, the pluripotent or induced pluripotent stem cells are hematopoietic stem cells.

In one aspect, cells that comprise a nucleus containing a genetic modification as described herein are also provided, e.g., a modification introduced into a cell by pronuclear injection. In one embodiment, the pluripotent, induced pluripotent, or totipotent stem cells comprise a genetically modified immunoglobulin genomic locus, wherein the genomic locus comprises, from 5' to 3', (1) an FRT recombination site, (2) human V_H gene segments, (3) a mouse adam6 gene, (4) a loxP recombination site, (5) histidine-substituted human D gene segments, (6) human J_H gene segments, followed by (7) a mouse E_i (intronic enhancer), and (8) a mouse IgM constant region nucleotide sequence.

In one aspect, a lymphocyte isolated from a genetically modified non-human animal as described herein is provided. In one embodiment, the lymphocyte is a B cell, wherein the B cell comprises an immunoglobulin genomic locus comprising an unrearranged heavy chain variable region nucleotide sequence wherein the unrearranged heavy chain variable gene sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon.

In one aspect, a lymphocyte isolated from a genetically modified non-human animal as described herein is provided. In one embodiment, the lymphocyte is a B cell, wherein the B cell comprises an immunoglobulin locus that comprises a human V, D, and J gene segment, wherein at least one of the human D gene segment has been inverted 5' to 3' with respect to wild-type sequences, and wherein at least one reading frame of the inverted human D gene segment encodes at least one histidine residue. In one embodiment, the B cell is capable of producing an antigen-binding protein comprising the genetically modified heavy chain variable domain as described herein. In one embodiment, the genetically modified heavy chain variable domain as described herein is operably linked to a heavy chain constant region amino acid sequence.

In one aspect, a B cell population is provided that are capable of expressing an antigen-binding protein comprising at least one histidine residue in a heavy chain variable domain, wherein the B cell population comprises any genetic modifications as described herein. In one embodiment, the at least one histidine residue is present in a heavy chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the at least one histidine residue is present in CDR3.

In one aspect, a B cell population is provided that are capable of expressing an antigen-binding protein with enhanced serum half-life and/or enhanced pH-dependent recyclability, wherein the B cell population comprises any genetic modifications as described herein.

In one aspect, a method for making a non-human animal comprising a genetically modified immunoglobulin heavy chain variable locus is provided, comprising:

(a) modifying a genome of a non-human animal to delete or render non-functional endogenous immunoglobulin heavy chain V, D, and J gene segments (e.g., via insertion of a nucleotide sequence, e.g., an exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement or inversion of endogenous V_H, D, J_H segments); and

(b) placing in the genome an unrearranged heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon as described herein.

In one embodiment, the non-human animal is a mammal, including a rodent, e.g., a mouse, a rat, or a hamster.

In one embodiment, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of the endogenous non-histidine codons are replaced with histidine codons.

In one embodiment, the endogenous non-histone codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

In one embodiment, the added or substituted histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes an immunoglobulin variable domain selected from an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, a combination thereof.

In one embodiment, the added substituted histidine codon histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a complementary determining region (CDR) selected from a CDR1, a CDR2, a CDR3, and a combination thereof.

In one embodiment, the added or substituted histidine codon is present in an unrearranged heavy chain variable

region nucleotide sequence that encodes a frame region (FR) selected from FR1, FR2, FR3, FR4, and a combination thereof.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human V_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human V_H gene segment has been replaced with a histidine codon.

In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human V_H gene segment with a histidine codon, wherein the human V_H gene segment is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, and a combination thereof.

In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a genetically modified human J_H gene segment, wherein one or more endogenous non-histidine codon in at least one reading frame of the human J_H gene segment has been replaced with a histidine codon.

In one embodiment, the human unrearranged heavy chain variable region nucleotide sequence comprises a modification that replaces at least one endogenous non-histidine codon of a human J_H segment with a histidine codon, wherein the human J_H gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, and a combination thereof.

In one embodiment, the added or substituted histidine codon is present in a heavy chain variable region nucleotide sequence that encodes part of a CDR3. In one embodiment, the part of CDR3 comprises an amino acid sequence derived from a reading frame of a genetically modified human D gene segment comprising a modification that replaces at least one endogenous non-histidine codon in the reading frame with a histidine codon.

In one embodiment, the endogenous non-histidine codon that is substituted with a histidine codon encodes the amino acid selected from Y, N, D, Q, S, W, and R.

In one embodiment, the added or substituted histidine codon is present in at least one reading frame of the human D gene segment that is most frequently observed in VELOCIMMUNE® humanized immunoglobulin mice.

In one embodiment, the reading frame of the genetically modified human D gene segment that encodes part of CDR3 is selected from a hydrophobic frame, a stop frame, and a hydrophilic frame.

In one embodiment, the reading frame is a hydrophobic frame of a human D gene segment.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (GTTGT; SEQ ID NO: 88), D1-7 (GITGT; SEQ ID NO: 89), D1-20 (GITGT; SEQ ID NO: 89), and D1-26 (GIVGAT; SEQ ID NO:90), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (DIVVVPAAI; SEQ ID NO:92), D2-8 (DIVLMVYAI; SEQ ID NO: 94), D2-15 (DIVVVVAAT; SEQ ID NO:95), and D2-21 (HIVVVTAI; SEQ ID NO: 97), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (ITIFGVVII; SEQ ID NO:98), D3-9 (ITIF*LVII; SEQ ID NO:99, SEQ ID NO:100), D3-10 (ITMVRGVII; SEQ ID NO:101), D3-16 (IMITFGGVIVI; SEQ ID NO:102), and D3-22 (ITMIVVVIT; SEQ ID NO:103), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (TTVT; SEQ ID NO:105), D4-11 (TTVT; SEQ ID NO:105), D4-17 (TTVT; SEQ ID NO:105), D4-23 (TTVVT; SEQ ID NO: 106) and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (VDTAMV; SEQ ID NO: 107), D5-12 (VDIVATI; SEQ ID NO:108), D5-18 (VDTAMV; SEQ ID NO:107), and D5-24 (VEMATI; SEQ ID NO:109), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (SIAAR; SEQ ID NO:111), D6-13 (GIAAAG; SEQ ID NO:113), and D6-19 (GIAVAG; SEQ ID NO:115), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the hydrophobic frame comprises a nucleotide sequence that encodes human D7-27 (LTG), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the reading frame is a stop reading frame of a human D gene segment.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (VQLER; SEQ ID NO:8), D1-7 (V*LEL), D1-20 (V*LER), D1-26 (V*WELL; SEQ ID NO:12), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (RIL**YQLLY; SEQ ID NO:14), D2-8 (RILY*WCMLY; SEQ ID NO:16 and SEQ ID NO: 17), D2-15 (RIL*WW*LLL), and D2-21 (SILWW*LLF; SEQ ID NO:19), and the human D gene segment further comprises a

modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (VLRFLEWLLY; SEQ ID NO:21), D3-9 (VLRYFDWLL*; SEQ ID NO:23), D3-10 (VLLWFGELL*; SEQ ID NO:25), D3-16 (VL*LRLGELSLY; SEQ ID NO:27), and D3-22 (VLL***WLLL; SEQ ID NO:29), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (*LQ*L), D4-11 (*LQ*L), D4-17 (*LR*L), and D4-23 (*LRW*L), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (WIQLWL; SEQ ID NO:35); D5-12 (WI*WLRL; SEQ ID NO:37), D5-18 (WIQLWL; SEQ ID NO:35), and D5-24 (*RWLQL; SEQ ID NO:39), and the human D gene segment comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (V*QLV), D6-13 (V*QQLV; SEQ ID NO:41), and D6-19 (V*QWLV; SEQ ID NO:43), and the human D gene segment further comprises a modification that replaces at least one endogenous non-histidine codon in the nucleotide sequence with a histidine codon.

In one embodiment, the stop reading frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (*LG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon of the human D gene segment in the nucleotide sequence with a histidine codon.

In one embodiment, the reading frame is a hydrophilic frame of a human D gene segment.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D1-1 (YNWND; SEQ ID NO: 45), D1-7 (YNWNY; SEQ ID NO: 47), D1-20 (YNWND; SEQ ID NO: 45), and D1-26 (YSGSYY; SEQ ID NO:49), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D2-2 (GYCSSTSCYT; SEQ ID NO:51), D2-8 (GYCTNGVCYT; SEQ ID NO: 53), D2-15 (GYCSGGSCYS; SEQ ID NO:55), and D2-21 (AYCGGDCYS; SEQ ID NO:57), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D3-3 (YYDFWSGYYT; SEQ ID NO:59), D3-9 (YYDILTGYYN; SEQ ID NO:61), D3-10 (YYYGSGSYYN; SEQ ID NO:63), D3-16 (YYDYVWGSYRYT; SEQ ID NO:65), and D3-22 (YYYDSSGYYY; SEQ ID NO:67), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D4-4 (DYSNY; SEQ ID NO:69), D4-11 (DYSNY; SEQ ID NO:69), D4-17 (DYGDY; SEQ ID NO:71), and D4-23 (DYGGNS; SEQ ID NO:73), and the human D gene segment comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D5-5 (GYSYGY; SEQ ID NO:75), D5-12 (GYSGYDY; SEQ ID NO:77), D5-18 (GYSYGY; SEQ ID NO:75), and D5-24 (RDGYNY; SEQ ID NO:79), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of D6-6 (EYSSSS; SEQ ID NO: 81), D6-13 (GYSSSWY; SEQ ID NO:83), and D6-19 (GYSSGWY; SEQ ID NO:85), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence with a histidine codon. In one embodiment, the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 76, and a combination thereof.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes D7-27 (NWG), and the human D gene segment further comprises a modification that replaces at least one endogenous codon in the nucleotide sequence a histidine codon.

In one embodiment, the hydrophilic frame of the human D gene segment comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72,

SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, and a combination thereof.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (i.e., C_H1-hinge-C_H2-C_H3).

In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., L/R/S/P/Q or K) and/or 434 (e.g., H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (e.g., 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (e.g., M428L) and 434S (e.g., N434S) modification; a 428L, 259I (e.g., V259I), and 308F (e.g., V308F) modification; a 433K (e.g., H433K) and a 434 (e.g., 434Y) modification; a 252, 254, and 256 (e.g., 252Y, 254T, and 256E) modification; a 250Q and 428L modification (e.g., T250Q and M428L); and a 307 and/or 308 modification (e.g., 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence (e.g., an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, e.g., about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, e.g., at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

In one embodiment, the genetically modified locus comprises a modification that deletes or renders non-functional all or substantially all endogenous V_H, D, and J_H gene segments; and the genomic locus comprises the genetically modified, unrearranged human heavy chain variable region nucleotide sequence comprising a substitution of at least one endogenous non-histidine codon with a histidine codon in at least one reading frame. In one embodiment, the genetically modified, unrearranged immunoglobulin heavy chain variable gene sequence is present at an endogenous location (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome), or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome.

In one embodiment, the genetically modified locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

In one embodiment, the genetically modified locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment the mouse immunoglobulin light chain locus is a mouse λ locus.

In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (e.g., at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about

4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

In one embodiment, the genetically modified immunoglobulin locus described herein comprises a B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, e.g., antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

In one aspect, a method for making a non-human animal comprising a genetically modified immunoglobulin heavy chain variable locus is provided, comprising:

(a) modifying a genome of a non-human animal to delete or render non-functional endogenous immunoglobulin heavy chain V, D, and J gene segments (e.g., via insertion of a nucleotide sequence (e.g., an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement or inversion of endogenous V_H, D, J_H segments); and

(b) placing in the genome a human V_H, D, and J_H gene segment, wherein at least one of the human D gene segment has been inverted 5' to 3' with respect to a corresponding wild-type sequence, and wherein at least one reading frame of the inverted human D gene segment comprises a histidine codon.

In one embodiment, the non-human animal is a mammal, including a rodent, e.g., a mouse, a rat, or a hamster

In one embodiment, the genetically modified immunoglobulin locus is present in a germline genome.

In one embodiment, the genetically modified immunoglobulin locus encodes an immunoglobulin heavy chain variable domain comprising one or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, or 34 or more of histidine residues.

In one embodiment, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty one, at least twenty two, at least twenty three, at least twenty four, or all or substantially all of functional human D gene segments have inverted orientation with respect to corresponding wild type sequences.

In one embodiment, all or substantially all of endogenous immunoglobulin V_H, D, J_H gene segments are deleted from the immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence, e.g., exogenous nucleotide sequence, in the immunoglobulin locus or via non-functional rearrangement or inversion of all, or substantially all, endogenous immunoglobulin V_H, D, J_H segments), and the genetically modified immunoglobulin locus comprises a human V_H, D, and J_H gene segments, wherein at least one of the human D gene segment is present in an inverted orientation with respect to a corresponding wild type sequence, and wherein at least one reading frame in the inverted human D gene segment comprises at least one histidine codon.

In one embodiment, the inverted human D gene segment is operably linked to a human V_H gene segment, and/or human J_H gene segment

In one embodiment, the human D gene segment that is present in the inverted orientation relative to wild type sequences is selected from the group consisting of D1-1, D1-7, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-5, D5-12, D5-18, D5-24, D6-6, D6-13, D6-19, D7-27, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D1 gene segment selected from the group consisting of D1-1, D1-7, D1-20, D1-26, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D2 gene segment selected from the group consisting of D2-2, D2-8, D2-15, D2-21, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D3 gene segment selected from the group consisting of D3-3, D3-9, D3-10, D3-16, D3-22, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D4 gene segment selected from the group consisting of D4-4, D4-11, D4-17, D4-23, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D5 gene segment selected from the group consisting of D5-5, D5-12, D5-18, D5-24, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is a D6 gene segment selected from the group consisting of D6-6, D6-13, D6-19, and a combination thereof.

In one embodiment, the human D gene segment that is present in the inverted orientation relative to a corresponding wild type sequence is D7-27.

In one embodiment, the reading frame of the human D gene segment is selected from a stop reading frame, a hydrophilic reading frame, a hydrophobic reading frame, and a combination thereof.

In one embodiment, the unrearranged heavy chain variable region nucleotide sequence comprising the inverted human D gene segment is operably linked to a human or non-human heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA.

In one embodiment, the human unrearranged immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. In one embodiment, the heavy chain constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (i.e., C_H1-hinge-C_H2-C_H3).

In one embodiment, a heavy chain constant region nucleotide sequence is present at an endogenous locus (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., L/R/S/P/Q or K) and/or 434 (e.g., H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (e.g., 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (e.g., M428L) and 434S (e.g., N434S) modification; a 428L, 259I (e.g., V259I), and 308F (e.g., V308F) modification; a 433K (e.g., H433K) and a 434 (e.g., 434Y) modification; a 252, 254, and 256 (e.g., 252Y, 254T, and 256E) modification; a 250Q and 428L modification (e.g., T250Q and M428L); and a 307 and/or 308 modification (e.g., 308F or 308P), wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, N434S, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising an N434A mutation.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of T250Q, M248L, or both.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human heavy chain constant region amino acid sequence comprising a mutation selected from the group consisting of H433K, N434Y, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises: (1) a first allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a first heavy chain constant region nucleotide sequence encoding a first CH_3 amino acid sequence of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and (2) a second allele, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a second heavy chain constant region nucleotide sequence encoding a second C_H3 amino acid sequence of the human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, and wherein the second CH_3 amino acid sequence comprises a modification that reduces or eliminates binding for the second CH_3 amino acid sequence to Protein A (see, for example, US 2010/0331527A1, which is incorporated by reference herein in its entirety).

In one embodiment, the second CH_3 amino acid sequence comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In one embodiment the second CH_3 amino acid sequence further comprises an Y96F modification (by IMGT exon numbering; H436F by EU). In another embodiment, the second CH_3 amino acid sequence comprises both an H95R modification (by IMGT exon numbering; H435R by EU numbering) and an Y96F modification (by IMGT exon numbering; H436F by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG1 and further comprises a mutation selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L38M, N384S, K392N, V397M, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG2 and further comprises a mutation selected from the group consisting of N44S, K52N, and V82I (IMGT: N384S, K392N, and V422I by EU).

In one embodiment, the second CH_3 amino acid sequence is from a modified human IgG4 and further comprises a

mutation selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT: Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, all or substantially all endogenous V_H, D, and J_H gene segments are deleted from an immunoglobulin heavy chain locus or rendered non-functional (e.g., via insertion of a nucleotide sequence (e.g., an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement, or inversion, of the endogenous V_H, D, J_H segments). In one embodiment, e.g., about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous V_H, D, or J_H gene segments are deleted or rendered non-functional. In one embodiment, e.g., at least 95%, 96%, 97%, 98%, or 99% of endogenous functional V, D, or J gene segments are deleted or rendered non-functional.

In one embodiment, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders, all or substantially all, non-functional endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising at least one inverted human D gene segment as described herein wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the genetically modified immunoglobulin locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification does not affect the expression and/or function of the endogenous Adam6a gene, Adam6b gene, or both.

In one embodiment, the genetically modified immunoglobulin locus comprises an ectopically present Adam6a gene, Adam6b gene, or both. In one embodiment, the Adam6a gene is a non-human Adam6a gene. In one embodiment, the Adam6a gene is a mouse Adam6a gene. In one embodiment, the Adam6a gene is a human Adam6a gene. In one embodiment, the Adam6b gene is a non-human Adam6b gene. In one embodiment, the Adam6b gene is a mouse Adam6b gene. In one embodiment, the Adam6b gene is a human Adam6b gene.

In one embodiment, the genetically modified immunoglobulin locus further comprises a humanized, unrearranged λ and/or κ light chain variable gene sequence. In one embodiment, the humanized, unrearranged λ and/or κ light chain variable gene sequence is operably linked to an immunoglobulin light chain constant region nucleotide sequence selected from a λ light chain constant region nucleotide sequence and a κ light chain constant region nucleotide sequence. In one embodiment, the humanized, unrearranged λ light chain variable region nucleotide sequence is operably linked to a λ light chain constant region nucleotide sequence. In one embodiment, the λ light chain constant region nucleotide sequence is a mouse, rat, or human sequence. In one embodiment, the humanized, unrearranged κ light chain variable region nucleotide sequence is operably linked to a κ light chain constant region nucleotide sequence. In one embodiment, the κ light chain constant region nucleotide sequence is a mouse, rat, or human sequence.

In one embodiment, the genetically modified immunoglobulin locus comprises an unrearranged light chain variable gene sequence that contains at least one modification that introduces at least one histidine codon in at least one reading frame encoding a light chain variable domain. In one embodiment, the genetically modified immunoglobulin locus comprises a rearranged (e.g., a rearranged λ or κ V/J sequence) sequence that comprises one, two, three, or four codons for histidine in a light chain CDR. In one embodiment, the CDR is a selected from a CDR1, CDR2, CDR3, and a combination thereof. In one embodiment, the unrearranged or rearranged light chain variable region nucleotide sequence is an unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence. In one embodiment, the unrearranged or rearranged human λ or κ light chain variable region nucleotide sequence is present at an endogenous mouse immunoglobulin light chain locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse κ locus. In one embodiment, the mouse immunoglobulin light chain locus is a mouse immunoglobulin light chain locus is a mouse λ locus.

In one embodiment, the genetically modified immunoglobulin locus as described herein is present in an immunoglobulin heavy chain locus of a mouse. In one embodiment, the genetically modified immunoglobulin locus is present in a humanized immunoglobulin heavy chain locus in a VELOCIMMUNE® mouse.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein exhibits a weaker antigen binding at an acidic environment (e.g., at a pH of about 5.5 to about 6.0) than a corresponding wild-type heavy chain variable domain without the genetic modification.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at

an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

In one embodiment, the genetically modified immunoglobulin locus described herein comprises an enriched B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, e.g., antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein, when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

In one aspect, a method for making a non-human animal that is capable of producing an immunoglobulin heavy chain variable domain with enhanced serum half-life and/or enhanced pH-dependent recyclability is provided, comprising

(a) modifying a genome of a non-human animal to delete or render non-functional endogenous immunoglobulin heavy chain V, D, and J gene segments (e.g., via insertion of a nucleotide sequence (e.g., an exogenous nucleotide sequence) in the immunoglobulin locus or via non-functional rearrangement or inversion of endogenous V_H, D, J_H segments); and

(b) placing in the genome an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon, and wherein an antigen-binding protein comprising the immunoglobulin heavy chain variable domain produced by the non-human animal exhibits enhanced serum half-life and/or enhanced pH-dependent recyclability as compared to a wild-type immunoglobulin heavy chain domain.

In one embodiment, the non-human animal, upon contact with an antigen, can produce an enriched population of B cell repertoire that expresses an antigen-binding protein with enhanced serum half-life and/or enhanced pH-dependent recyclability, wherein the enriched B cell population comprises any genetic modifications as described herein.

In one embodiment, an antigen-binding protein produced by the genetically modified non-human animal is characterized by sufficient affinity to an antigen of interest at a neutral pH (e.g., pH of about 7.0 to about 7.4) and enhanced dissociation of the antibody from an antigen-antigen-binding protein complex at a pH less than the neutral pH (e.g., at an endosomal pH, e.g. pH of about 5.5 to 6.0).

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein is characterized by improved pH-dependent recyclability, enhanced serum half-life, or both as compared with a wild-type antigen-binding protein without the genetic modification.

In one embodiment, the genetically modified immunoglobulin locus described herein comprises a an enriched B cell population that, upon stimulation with an antigen of interest, is capable of producing antigen-binding proteins, e.g., antibodies, comprising a heavy chain variable domain comprising one or more histidine residues. The antigen-binding proteins as described herein when administered into a subject, exhibits an increased serum half-life over a corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain. In some embodiments, the antigen-binding protein described herein exhibits an increased serum half-life that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold higher than the corresponding wild-type antigen-binding protein, which possesses a similar or sufficiently similar amino acid sequence that encodes the heavy chain variable domain but does not comprise a histidine residue in the heavy chain variable domain.

In one embodiment, the antigen-binding protein comprises an immunoglobulin heavy chain variable domain that is capable of specifically binding an antigen of interest with an affinity (K_D) lower than 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, and 10^{-12} at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one aspect, a method for obtaining an antigen-binding protein with enhanced recyclability and/or improved serum half-life is provided, comprising:

(a) immunizing a non-human animal having a genetically modified immunoglobulin locus as described herein wherein the non-human animal comprises an unrearranged human heavy chain variable region nucleotide sequence comprising

an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon;

(b) allowing the non-human animal to mount an immune response;

(c) harvesting a lymphocyte (e.g., a B cell) from the immunized non-human animal;

(d) fusing the lymphocyte with a myeloma cell to form a hybridoma cell, and

(e) obtaining an antigen-binding protein produced by the hybridoma cell, wherein the antigen-binding protein exhibits enhanced recyclability and/or serum stability.

In one aspect, a genetically modified immunoglobulin heavy chain locus obtainable by any of the methods as described herein is provided.

In one aspect, a genetically modified non-human animal obtainable by any of the methods as described herein is provided.

In various embodiments, the non-human animal is a mammal. In one embodiment, the mammal is a rodent, e.g., a mouse, a rat, or a hamster.

In various embodiments, the genetically modified immunoglobulin loci as described herein are present in the germline genome of a non-human animal, e.g., a mammal, e.g., a rodent, e.g., a mouse, a rat, or a hamster.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1**A and **1**B illustrate the amino acid sequences encoded by the three reading frames (i.e., stop, hydrophilic, and hydrophobic reading frames) of human D gene segments (D) and the amino acid sequences encoded by the three reading frames of histidine-substituted human D gene segments (HD). Introduction of histidine codons (typed in bold) in the hydrophilic reading frame also changed many stop codons in the stop reading frame to Ser codons (typed in bold) but introduced few changes in the hydrophobic reading frame. The "*" symbol represents a stop codon, and the comma between the two SEQ ID NOs indicates that there are two amino acid sequences separated by the stop codon.

FIG. **2** illustrates schemes for targeting pLMa0174 containing a spectinomycin selection cassette into the 5' end of MAID 1116 (Step 1. BHR (Spec)). In Step 1, a chloramphenicol selection cassette, a neomycin selection cassette, a loxP site, two V_H gene segments (hV_H1-3 and hV_H1-2), the human Adam6 gene, all of which are located upstream of hV_H6-1, were deleted from the clone and replaced by a spectinomycin cassette to yield the V1433 clone. In Step 2 (BHR (Hyg+ Spec)), pNTu0002 containing a hygromycin cassette flanked by FRT sites was targeted into a region comprising human immunoglobulin D gene segments. Via Step 2, all human D gene segments were deleted from V1433 and replaced with the hygromycin cassette to yield MAID6011 VI 434 (clone 1).

FIG. **3** illustrates schemes for assembling histidine-substituted human D gene segments via sequential ligation.

FIG. **4** illustrates the introduction of pre-assembled, histidine-substituted human D gene segments containing a neomycin cassette into a region between the most D-proximal V_H gene segment (V_H6-1) and the most D-proximal J_H gene segment (J_H1) via enzyme-mediated digestion (PI-SceI and I-CeuI) and ligation. This process removes the hygromycin cassette from MAID 6011 V1434 and introduces pre-assembled human histidine-substituted D gene segments into the clone. Bacterial cells comprising a successfully targeted clone are selected based on both neomycin and spectinomycin resistance. The resulting clone (MAID6012 V1469) comprises, from 5' to 3', (1) a spectinomycin selection cassette, (2) a 50 kb arm comprising a human V_H gene segment (V_H6-1), (3) a neomycin cassette flanked by loxP sites, (4) human D gene segments containing histidine substitutions (HD 1.1-6.6 (9586 bp; SEQ ID NO: 1), HD 1.7-6.13 (9268 bp; SEQ ID NO: 2), HD 1.14-6.19 (9441 bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592 bp; SEQ ID NO: 4)), (5) about 25 kb of a genomic region containing human J_H gene segments, (6) a mouse E, sequence (SEQ ID NO: 5; an intronic enhancer that promotes V_H to DJ_H rearrangement in developing B cells), and (7) a mouse IgM constant region nucleotide sequence (mIgM exon 1; SEQ ID NO: 7).

FIG. **5** illustrates schemes for deleting the human immunoglobulin heavy chain D gene region from the MAID 1460 heterozygous ES cells by targeting the 129 strain-derived chromosome of MAID 1460 het with the hygromycin selection cassette in MAID 6011 V1434.

FIG. **6** shows a list of primers and probes used to confirm a loss of allele (LOA), a gain of allele (GOA), or a parental allele (Parental) in the screening assays for identifying MAID 6011.

FIG. **7** illustrates schemes for constructing MAID 6012 het by targeting MAID 6011 heterozygous ES cells with MAID 6012 V1469. Electroporation of the MAID 6012 V1469 construct into the MAID 6011 heterozygous ES cells yielded MAID 6012 heterozygous ES cells in which the 129 strain-derived chromosome is modified to contain, from 5' to 3' direction, an FRT site, human V_H gene segments, a mouse genomic region comprising adam6 genes, a floxed neomycin selection cassette, human D gene segments comprising histidine substitutions (HD 1.1-6.6 (9586 bp; SEQ ID NO: 1), HD 1.7-6.13 (9268 bp; SEQ ID NO: 2), HD 1.14-6.19 (9441 bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592 bp; SEQ ID NO: 4)), human J_H gene segments, a mouse E_i sequence (SEQ ID NO: 5; an intronic enhancer that promotes V_H to DJ_H rearrangement in developing B cells), and a mouse IgM constant region nucleotide sequence (mIgM exon 1; SEQ ID NO: 7).

FIG. **8** shows a list of primers and probes used to confirm a loss of allele (LOA), a gain of allele (GOA), or a parental allele (Parental) in the screening assay for identifying MAID 6012.

FIG. **9** illustrates schemes for removing a neomycin cassette from MAID 6012 heterozygous ES cells. Electroporation of a Cre-expressing plasmid into the MAID 6012 ES cells lead to recombination and deletion of the floxed neomycin cassette, yielding MAID 6013 heterozygous ES cells.

FIGS. **10**A-**10**E illustrate human D gene segment nucleotide sequences with translations for each of the six reading frames, i.e., three reading frames for direct 5' to 3' orientation and three reading frames for inverted orientation (3' to 5' orientation). The "*" symbol represents a stop codon, and the comma between two SEQ ID NOs indicates that there are two amino acid sequences separated by the stop codon.

FIGS. **11**-**13** illustrate mRNA sequences and their encoded protein sequences expressed by 6013 F0 heterozygous mice, which comprise histidine-substituted human D gene segments (HD 1.1-6.6 (9586 bp; SEQ ID NO: 1), HD 1.7-6.13 (9268 bp; SEQ ID NO: 2), HD 1.14-6.19 (9441 bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592 bp; SEQ ID NO: 4)) in the immunoglobulin heavy chain locus in their 129 strain-derived chromosome. The boxed sequences in each figure indicate the presence of histidine codons in the CDR3 sequences derived from the genetically modified immunoglobulin heavy chain locus comprising the histidine-substituted human D gene segments. FWR represents frame region and CDR represents complementarity determining region. In the

alignment, the dot "." indicates a sequence identical to the query sequence, and the dash "-" indicates a gap in the sequence.

FIG. **14** illustrates histidine incorporation frequency in immunoglobulin heavy chain CDR3 sequences. The X-axis represents the number of histidine codons appeared in each CDR3 sequence, and the Y-axis represents the corresponding proportion of reads. The "6013 F0 het" indicates CDR3 sequences expressed by the 6013 heterozygous mice comprising histidine-substituted D gene segments. The "V13-Adam6" indicates CDR3 sequences obtained from control mice comprising human V_H, D, and J_H gene segments without the histidine modification as described herein. The "ASAP" indicates CDR3 sequences obtained from the Regeneron antibody database, which was used as another control.

DETAILED DESCRIPTION OF THE INVENTION

This invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention is defined by the claims.

Unless defined otherwise, all terms and phrases used herein include the meanings that the terms and phrases have attained in the art, unless the contrary is clearly indicated or clearly apparent from the context in which the term or phrase is used. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, particular methods and materials are now described. All publications mentioned are hereby incorporated by reference

DEFINITIONS

The term "complementary determining region" or "CDR," as used herein, includes an amino acid sequence encoded by a nucleic acid sequence of an organism's immunoglobulin genes that normally (i.e., in a wild type animal) appears between two framework regions in a variable region of a light or a heavy chain of an immunoglobulin molecule (e.g., an antibody or a T cell receptor). A CDR can be encoded by, for example, a germline sequence or a rearranged sequence, and, for example, by a naïve or a mature B cell or a T cell. A CDR can be somatically mutated (e.g., vary from a sequence encoded in an animal's germline), humanized, and/or modified with amino acid substitutions, additions, or deletions. In some circumstances (e.g., for a CDR3), CDRs can be encoded by two or more sequences (e.g., germline sequences) that are not contiguous (e.g., in an unrearranged nucleic acid sequence) but are contiguous in a B cell nucleic acid sequence, e.g., as a result of splicing or connecting the sequences (e.g., V-D-J recombination to form a heavy chain CDR3).

The term "dissociative half-life" or "$t_{1/2}$" as used herein refers to the value calculated by the following formula: $t_{1/2}$ (min)=(In2/k_d)/60, wherein k_d represents a dissociation rate constant.

The term "germline" in reference to an immunoglobulin nucleic acid sequence includes a nucleic acid sequence that can be passed to progeny.

The phrase "heavy chain," or "immunoglobulin heavy chain" includes an immunoglobulin heavy chain sequence, including immunoglobulin heavy chain constant region sequence, from any organism. Heavy chain variable domains include three heavy chain CDRs and four FR regions, unless otherwise specified. Fragments of heavy chains include CDRs, CDRs and FRs, and combinations thereof. A typical heavy chain has, following the variable domain (from N-terminal to C-terminal), a C_H1 domain, a hinge, a C_H2 domain, and a C_H3 domain. A functional fragment of a heavy chain includes a fragment that is capable of specifically recognizing an epitope (e.g., recognizing the epitope with a K_D in the micromolar, nanomolar, or picomolar range), that is capable of expressing and secreting from a cell, and that comprises at least one CDR. Heavy chain variable domains are encoded by variable region nucleotide sequence, which generally comprises V_H, D_H, and J_H segments derived from a repertoire of V_H, D_H, and J_H segments present in the germline. Sequences, locations and nomenclature for V, D, and J heavy chain segments for various organisms can be found in IMGT database, which is accessible via the internet on the world wide web (www) at the URL "imgt.org."

The phrase "light chain" includes an immunoglobulin light chain sequence from any organism, and unless otherwise specified includes human kappa (κ) and lambda (λ) light chains and a VpreB, as well as surrogate light chains. Light chain variable domains typically include three light chain CDRs and four framework (FR) regions, unless otherwise specified. Generally, a full-length light chain includes, from amino terminus to carboxyl terminus, a variable domain that includes FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, and a light chain constant region amino acid sequence. Light chain variable domains are encoded by the light chain variable region nucleotide sequence, which generally comprises light chain V_L and light chain J_L gene segments, derived from a repertoire of light chain V and J gene segments present in the germline. Sequences, locations and nomenclature for light chain V and J gene segments for various organisms can be found in IMGT database, which is accessible via the internet on the world wide web (www) at the URL "imgt.org." Light chains include those, e.g., that do not selectively bind either a first or a second epitope selectively bound by the epitope-binding protein in which they appear. Light chains also include those that bind and recognize, or assist the heavy chain with binding and recognizing, one or more epitopes selectively bound by the epitope-binding protein in which they appear.

The phrase "operably linked" refers to a relationship wherein the components operably linked function in their intended manner. In one instance, a nucleic acid sequence encoding a protein may be operably linked to regulatory sequences (e.g., promoter, enhancer, silencer sequence, etc.) so as to retain proper transcriptional regulation. In one instance, a nucleic acid sequence of an immunoglobulin variable region (or V(D)J segments) may be operably linked to a nucleic acid sequence of an immunoglobulin constant region so as to allow proper recombination between the sequences into an immunoglobulin heavy or light chain sequence.

The phrase "somatically mutated," as used herein, includes reference to a nucleic acid sequence from a B cell that has undergone class-switching, wherein the nucleic acid sequence of an immunoglobulin variable region, e.g., a heavy chain variable region (e.g., a heavy chain variable domain or including a heavy chain CDR or FR sequence) in the class-switched B cell is not identical to the nucleic acid sequence in the B cell prior to class-switching, such as, for example a difference in a CDR or a framework nucleic acid sequence between a B cell that has not undergone class-switching and a B cell that has undergone class-switching. The phrase "somatically mutated" includes reference to nucleic acid sequences from affinity-matured B cells that are not identical to corresponding immunoglobulin variable region nucleotide

sequences in B cells that are not affinity-matured (i.e., sequences in the genome of germline cells). The phrase "somatically matured" also includes reference to an immunoglobulin variable region nucleic acid sequence from a B cell after exposure of the B cell to an epitope of interest, wherein the nucleic acid sequence differs from the corresponding nucleic acid sequence prior to exposure of the B cell to the epitope of interest. The term "somatically mutated" also refers to sequences from antibodies that have been generated in an animal, e.g., a mouse having human immunoglobulin variable region nucleic acid sequences, in response to an immunogen challenge, and that result from the selection processes inherently operative in such an animal.

Non-Human Animals that Express Immunoglobulin Heavy Chain Variable Domain Comprising Histidine Residues

The described invention provides genetically modified non-human animals that can produce antigen-binding proteins with pH-dependent antigen binding characteristics. In various embodiments, the antigen-binding proteins produced by the genetically modified non-human animals as described herein exhibit increased pH-dependent recycling efficiency and/or enhanced serum half-life. In particular, the described invention employs genetic modifications in the immunoglobulin heavy chain locus to introduce histidine codons into a human heavy chain variable region nucleotide sequence and, optionally, to introduce a mutation(s) in a constant region nucleotide sequence that encodes C_H2 and/or C_H3 domains that increases the binding of the antibody constant region to an FcRn receptor, which facilitates recycling of the antigen-binding protein. Antigen-binding proteins comprising the modification may more loosely bind its target in an acidic intracellular compartment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0) than in an extracellular environment or at the surface of a cell (i.e., at a physiological pH, e.g., a pH ranging from about 7.0 to about 7.4) due to protonated histidine residues located in the antigen binding sites. Therefore, the antigen-biding proteins comprising the genetic modifications as described herein would be able to be recycled more rapidly or efficiently than wild-type antigen-binding proteins that do not comprise such genetic modifications following target-mediated endocytosis. Furthermore, since the modified histidine residues are protonated only in an acidic environment, but not at a neutral pH, it is expected that such modification would not affect binding affinity and/or specificity of the antigen-binding protein toward an antigen of interest at a physiological pH.

In various aspects, non-human animals are provided comprising immunoglobulin heavy chain loci that comprise an unrearranged human heavy chain variable region nucleotide sequence, wherein the unrearranged human heavy chain variable region nucleotide sequence comprises an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon.

In various aspects, methods of making and using the non-human animals are also provided. When immunized with an antigen of interest, the genetically modified non-human animals are capable of generating B cell populations that produce antigen-binding proteins comprising heavy chain variable domains with histidine residues, wherein the antigen-binding proteins exhibit enhanced pH-dependent recycling and/or increased serum half-life. In various embodiments, the non-human animals generate B cell populations that express human heavy chain variable domains along with cognate human light chain variable domains. In various embodiments, the genetically modified immunoglobulin heavy chain loci are present in a germline genome of the non-human animal.

In various embodiments, the genetically modified immunoglobulin heavy chain locus comprises a modification that deletes or renders, all or substantially all, non-functional endogenous V_H, D, and J_H gene segments; and the genetically modified locus comprises an unrearranged heavy chain variable region nucleotide sequence comprising one or more human V_H, D, and/or J_H gene segments having one or more histidine codons, wherein the unrearranged heavy chain variable region nucleotide sequence is present at an endogenous location (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome). In one embodiment, e.g., about 80% or more, about 85% or more, about 90% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more of all endogenous heavy chain V, D, or J gene segments are deleted or rendered non-functional. In one embodiment, e.g., at least 95%, 96%, 97%, 98%, or 99% of endogenous functional heavy chain V, D, or J gene segments are deleted or rendered non-functional.

In one embodiment, the non-human animal is a mammal. Although embodiments directed to introducing histidine codons into an unrearranged human heavy chain variable gene sequence in a mouse are extensively discussed herein, other non-human animals are also provided that comprise a genetically modified immunoglobulin locus containing an unrearranged human heavy chain variable region nucleotide sequence comprising an addition of least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon. Such non-human animals include any of those which can be genetically modified to express the histidine-containing heavy chain variable domain as disclosed herein, including, e.g., mouse, rat, rabbit, pig, bovine (e.g., cow, bull, buffalo), deer, sheep, goat, chicken, cat, dog, ferret, primate (e.g., marmoset, rhesus monkey), etc. For example, for those non-human animals for which suitable genetically modifiable ES cells are not readily available, other methods are employed to make a non-human animal comprising the genetic modification. Such methods include, e.g., modifying a non-ES cell genome (e.g., a fibroblast or an induced pluripotent cell) and employing somatic cell nuclear transfer (SCNT) to transfer the genetically modified genome to a suitable cell, e.g., an enucleated oocyte, and gestating the modified cell (e.g., the modified oocyte) in a non-human animal under suitable conditions to form an embryo. Methods for modifying a non-human animal genome (e.g., a pig, cow, rodent, chicken, etc. genome) include, e.g., employing a zinc finger nuclease (ZFN) or a transcription activator-like effector nuclease (TALEN) to modify a genome to include a nucleotides sequence that encodes

In one embodiment, the non-human animal is a small mammal, e.g., of the superfamily Dipodoidea or Muroidea. In one embodiment, the genetically modified animal is a rodent. In one embodiment, the rodent is selected from a mouse, a rat, and a hamster. In one embodiment, the rodent is selected from the superfamily Muroidea. In one embodiment, the genetically modified animal is from a family selected from Calomyscidae (e.g., mouse-like hamsters), Cricetidae (e.g., hamster, New World rats and mice, voles), Muridae (true mice and rats, gerbils, spiny mice, crested rats), Nesomyidae (climbing mice, rock mice, with-tailed rats, Malagasy rats and mice), Platacanthomyidae (e.g., spiny dormice), and Spalacidae (e.g., mole rates, bamboo rats, and zokors). In a specific embodiment, the genetically modified rodent is selected from

a true mouse or rat (family Muridae), a gerbil, a spiny mouse, and a crested rat. In one embodiment, the genetically modified mouse is from a member of the family Muridae. In one embodiment, the animal is a rodent. In a specific embodiment, the rodent is selected from a mouse and a rat. In one embodiment, the non-human animal is a mouse.

In one embodiment, the non-human animal is a rodent that is a mouse of a C57BL strain selected from C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6N, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. In another embodiment, the mouse is a 129 strain. In one embodiment, the 129 strain is selected from the group consisting of 129P1, 129P2, 129P3, 129X1, 129S1 (e.g., 129S1/SV, 129S1/Svlm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2 (see, e.g., Festing et al. (1999) Revised nomenclature for strain 129 mice, Mammalian Genome 10:836, see also, Auerbach et al. (2000) Establishment and Chimera Analysis of 129/SvEv- and C57BL/6-Derived Mouse Embryonic Stem Cell Lines). In one embodiment, the genetically modified mouse is a mix of an aforementioned 129 strain and an aforementioned C57BL strain (e.g., a C57BL/6 strain). In another embodiment, the mouse is a mix of aforementioned 129 strains, or a mix of aforementioned C57BL/6 strains. In one embodiment, the 129 strain of the mix is a 129S6 (129/SvEvTac) strain. In another embodiment, the mouse is a mix of a 129/SvEv- and a C57BL/6-derived strain. In a specific embodiment, the mouse is a mix of a 129/SvEv- and a C57BL/6-derived strain as described in Auerbach et al. 2000 *Bio Techniques* 29:1024-1032. In another embodiment, the mouse is a BALB strain, e.g., BALB/c strain. In another embodiment, the mouse is a mix of a BALB strain (e.g., BALB/c strain) and another aforementioned strain.

In one embodiment, the non-human animal is a rat. In one embodiment, the rat is selected from a Wistar rat, an LEA strain, a Sprague Dawley strain, a Fischer strain, F344, F6, and Dark Agouti. In one embodiment, the rat strain is a mix of two or more of a strain selected from the group consisting of Wistar, LEA, Sprague Dawley, Fischer, F344, F6, and Dark Agouti.

In one embodiment, the non-human animal is a mouse. In one embodiment, the mouse is a VELOCIMMUNE® humanized mouse.

VELOCIMMUNE® humanized mice (see, e.g., U.S. Pat. No. 6,596,541, U.S. Pat. No. 7,105,348, and US20120322108A1, which are incorporated herein by reference in their entireties), which contain a precise replacement of mouse immunoglobulin variable regions with human immunoglobulin variable regions at the endogenous mouse loci, display a surprising and remarkable similarity to wild-type mice with respect to B cell development. VELOCIMMUNE® humanized mice display an essentially normal, wild-type response to immunization that differed only in one significant respect from wild-type mice—the variable regions generated in response to immunization are fully human.

VELOCIMMUNE® humanized mice contain a precise, large-scale replacement of germline variable region nucleotide sequences of mouse immunoglobulin heavy chain (IgH) and immunoglobulin light chain (e.g., κ light chain, Igκ) with corresponding human immunoglobulin variable region nucleotide sequences, at the endogenous loci (see, e.g., U.S. Pat. No. 6,596,541, U.S. Pat. No. 7,105,348, US 20120322108A1, which are incorporated herein by reference in their entireties). In total, about six megabases of mouse loci are replaced with about 1.5 megabases of human genomic sequence. This precise replacement results in a mouse with hybrid immunoglobulin loci that make heavy and light chains that have a human variable regions and a mouse constant region. The precise replacement of mouse V_H-D-J_H and Vκ-Jκ segments leave flanking mouse sequences intact and functional at the hybrid immunoglobulin loci. The humoral immune system of the mouse functions like that of a wild-type mouse. B cell development is unhindered in any significant respect and a rich diversity of human variable regions is generated in the mouse upon antigen challenge.

VELOCIMMUNE® humanized mice are possible because immunoglobulin gene segments for heavy and κ light chains rearrange similarly in humans and mice, which is not to say that their loci are the same or even nearly so—clearly they are not. However, the loci are similar enough that humanization of the heavy chain variable gene locus can be accomplished by replacing about three million base pairs of contiguous mouse sequence that contains all the V_H, D, and J_H gene segments with about one million bases of contiguous human genomic sequence covering basically the equivalent sequence from a human immunoglobulin locus.

In some embodiments, further replacement of certain mouse constant region nucleotide sequences with human constant region nucleotide sequences (e.g., replacement of mouse heavy chain C_H1 nucleotide sequence with human heavy chain C_H1 nucleotide sequence, and replacement of mouse light chain constant region nucleotide sequence with human light chain constant region nucleotide sequence) results in mice with hybrid immunoglobulin loci that make antibodies that have human variable regions and partly human constant regions, suitable for, e.g., making fully human antibody fragments, e.g., fully human Fab's. Mice with hybrid immunoglobulin loci exhibit normal variable gene segment rearrangement, normal somatic hypermutation frequencies, and normal class switching. These mice exhibit a humoral immune system that is indistinguishable from wild type mice, and display normal cell populations at all stages of B cell development and normal lymphoid organ structures—even where the mice lack a full repertoire of human variable region nucleotide segments. Immunizing these mice results in robust humoral responses that display a wide diversity of variable gene segment usage.

The precise replacement of the mouse germline variable region nucleotide sequence allows for making mice that have partly human immunoglobulin loci. Because the partly human immunoglobulin loci rearrange, hypermutate, and class switch normally, the partly human immunoglobulin loci generate antibodies in a mouse that comprise human variable regions. Nucleotide sequences that encode the variable regions can be identified and cloned, then fused (e.g., in an in vitro system) with any sequences of choice, e.g., any immunoglobulin isotype suitable for a particular use, resulting in an antibody or antigen-binding protein derived wholly from human sequences.

In various embodiments, at least one histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes an N-terminal region, a loop 4 region, a CDR1, a CDR2, a CDR3, or a combination thereof.

In various embodiments, at least one histidine codon is present in an unrearranged heavy chain variable region nucleotide sequence that encodes a framework region (FR) selected from the group consisting of FR1, FR2, FR3, and FR4.

In various aspects, the genetically modified immunoglobulin locus comprises a nucleotide sequence wherein at least one codon has been replaced with a histidine codon.

In various aspects, the genetically modified immunoglobulin locus comprises an unrearranged human heavy chain vari-

able region nucleotide sequence comprising a substitution of at least one endogenous non-histidine codon with a histidine codon.

In one embodiment, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, or 61 or more of the endogenous non-histidine codons are replaced with histidine codons.

Previous studies on reading frame usage of human immunoglobulin D gene segments have shown that, of the three reading frames (i.e., stop, hydrophobic, and hydrophilic), the stop frame is used very infrequently. Apparently, some stop frames are chewed back and result in expression. However, stop reading frames are used at such a low frequency that for the purposes of engineering histidine codons, it is more efficient not to use the stop reading frame. As between hydrophilic and hydrophobic reading frames, the hydrophilic reading frame appears to be preferred. Thus, in one embodiment, the hydrophilic reading frame of human D gene segments is engineered to contain one or more histidine codons (as compared with the stop frame or with the hydrophobic frame).

Methods of introducing a mutation in vitro, e.g., site-directed mutagenesis, are well known in the art. In some embodiments of the described invention, histidine codons are enriched by designing histidine-substituted human D gene segments in silico (e.g., mutation of Y, D, and N codons to H codons, e.g., CAT, CAC), which are synthesized (e.g., chemical synthesis) with (unique) restriction enzyme sites for ligating them back together. The synthesized D gene segments are made with the appropriate recombination signal sequences (RSS) upstream and downstream. In one embodiment, when ligated to one another, the synthesized histidine-substituted D gene segments include the intergenic sequences observed in a human between each D gene segment.

It is understood that the codons that encode the one or more histidines, upon rearrangement and/or somatic hypermutation, may change such that one or more of the histidines will be changed to another amino acid. However, this may not occur for each and every codon encoding histidine, in each and every rearrangement in the non-human animal. If such changes occur, the changes may occur in some but not all B cells or in some but not all heavy chain variable sequences.

In various aspects, the genetically modified immunoglobulin locus comprises a human heavy chain V, D, and J gene segment, wherein at least one of the human D gene segment has been inverted 5' to 3' with respect to a corresponding wild-type sequence, and wherein at least one reading frame of the inverted human D gene segment comprises a histidine codon.

In various embodiments, the nucleotide sequence comprises one or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, or 25 or more of histidine codons.

There are 25 functional human D gene segments in 6 families of 3-5 members each (one family—the D7 family—has a single member). Direct recombination of human D gene segments is much more frequent than inversion, although inverted reading frames exhibit more histidine codons. Certain D gene segments and reading frames are used more frequently than others. All three direct reading frames and all three inverted orientation reading frames for all the functional D gene segments are presented in FIGS. **10**A-**10**E. As shown in FIGS. **10**A-**10**E, there are many more histidine codons in inverted reading frames than in direct reading frames. More specifically, there are 34 histidines in inverted reading frames and only four in direct reading frames. In addition, of the four in direct reading frames, three histidines are encoded by pseudogenes or present in alternate alleles. Therefore, there is only a single direct reading frame of a germline human D gene segment that contains a histidine codon, with further histidine codons possibly encountered in alternate alleles (presumably in subsets of the human population).

Inverted D rearrangements are extremely rare. Tuaillon et al. (J. Immunol., 154(12): 5453-6465, incorporated by reference herein in its entirety) showed that usage of inverted reading frames (as measured by limiting dilution PCT) is very rare, i.e., that the ratio of direct to indirect rearrangements are, in most cases, 100 to 1000. To the extent that the ratio of direct to indirect rearrangement was low, it was only observed in those D segments that exhibit very low usage. It was also shown that D gene segment family 7, which is located adjacent to J1 (far down from other D family members) is mostly used in fetuses, but exhibits a low usage in adults (Schroeder et al., Immunology 30, 2006, 119-135, incorporated by reference herein in its entirety). Therefore, in one embodiment, D family 7 sequences are not inverted 5' to 3'.

In one embodiment, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty one, at least twenty two, at least twenty three, at least twenty four, or all or substantially all of the human functional D gene segments are inverted 5' to 3' with respect to corresponding wild type sequences.

In one embodiment, the human immunoglobulin heavy chain variable domain comprising at least one non-naturally occurring histidine residue exhibits pH-dependent antigen binding characteristics. For example, an antibody comprising the modified immunoglobulin heavy chain variable domain binds a target with sufficient affinity at around a neutral pH (e.g., pH of about 7.0 to about 7.4), but either does not bind or binds weaker to the same target at an acidic pH (e.g., pH of about 5.5 to about 6.0). In one embodiment, the acidic pH is selected from about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, and about 6.0. In one embodiment, the neutral pH is selected from about 7.0, about 7.1, about 7.2, about 7.3, and about 7.4.

In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 2 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than

1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 25° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin heavy chain locus as described herein has a dissociative half-life ($t_{1/2}$) of less than 1 min at an acidic pH (e.g., pH of about 5.5 to about 6.0) at 37° C. In one embodiment, an antigen-binding protein comprising a heavy chain variable domain expressed by the genetically modified immunoglobulin locus as described herein has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold decrease in dissociative half-life ($t_{1/2}$) at an acidic pH (e.g., pH of about 5.5 to about 6.0) as compared to the dissociative half-life ($t_{1/2}$) of the antigen-binding protein at a neutral pH (e.g., pH of about 7.0 to about 7.4).

In one embodiment, antigen binding proteins comprising the genetically modified human immunoglobulin heavy chain variable domain is capable of specifically binding an antigen of interest with an affinity (K_D) lower than 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} or 10^{-10}, 10^{-11}, 10^{-12} at a neutral or physiological pH (pH of about 7.0 to about 7.4).

The altered binding property of the immunoglobulin heavy chain variable domain at an acidic pH (e.g., pH of about 5.5 to about 6.0) would, in some circumstances, allow faster turnover of the antibody because the therapeutic antibody will bind a target on a cell's surface, be internalized into an endosome, and more readily or more rapidly dissociate from the target in the endosome, so that the therapeutic can be recycled to bind yet another molecule of target present in another cell. This would allow one to administer a therapeutic antibody at a lower dose, or administer the therapeutic antibody less frequently. This is particularly useful in a situation where it is not desirable to administer a therapeutic antibody frequently, or administer at a level above a certain dosage for safety or toxicity reasons.

In various embodiments, the human immunoglobulin heavy chain variable region nucleotide sequence as described herein is operably linked to a human or non-human heavy chain constant region nucleotide sequence (e.g., a heavy chain constant region nucleotide sequence that encodes an immunoglobulin isotype selected from IgM, IgD, IgG, IgE, and IgA). In various embodiments, the human or non-human heavy chain constant region nucleotide sequence is selected from the group consisting of a C_H1, a hinge, a C_{H2}, a C_{H3}, and a combination thereof. In one embodiment, the constant region nucleotide sequence comprises a C_H1, a hinge, a C_H2, and a C_H3 (e.g., C_H1-hinge-a C_H2-C_H3).

In various embodiments, the heavy chain constant region nucleotide sequence is present at an endogenous locus (i.e., where the nucleotide sequence is located in a wild-type non-human animal) or present ectopically (e.g., at a locus different from the endogenous immunoglobulin chain locus in its genome, or within its endogenous locus, e.g., within an immunoglobulin variable locus, wherein the endogenous locus is placed or moved to a different location in the genome).

In one embodiment, the heavy chain constant region nucleotide sequence comprises a modification in a C_H2 or a C_H3, wherein the modification increases the affinity of the heavy chain constant region amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

The neonatal Fc receptor for IgG (FcRn) has been well characterized in the transfer of passive humoral immunity from a mother to her fetus across the placenta and proximal small intestine (Roopenian, D. and Akilesh, S., Nat. Rev. Immun., 2007, 7:715-725, which is incorporated by reference herein in its entirety). FcRn binds to the Fc portion of IgG at a site that is distinct from the binding sites of the classical FcγRs or the Clq component of complement, which initiates the classical pathway of complement activation. More specifically, it was shown that FcRn binds the C_H2-C_H3 hinge region of IgG antibodies—a versatile region of Fc that also binds Staphylococcal protein A, Streptococcal protein G, and the rheumatoid factor. In contrast to other Fc-binding proteins, however, FcRn binds the Fc region of IgG in a strictly pH-dependent manner; at physiological pH 7.4, FcRn does not bind IgG, whereas at the acidic pH of the endosome (e.g., where the pH ranges from about 5.5 to about 6.0), FcRn exhibits a low micromolar to nanomolar affinity for the Fc region of IgG. This pH-dependent interaction has been shown to be mediated by the titration of histidine residues in the C_H2-C_H3 region of IgG and their subsequent interaction with acidic residue on the surface of FcRn (Roopenian, D. and Akilesh, S., Nat. Rev. Immun., 2007, 7:715-725, incorporated by reference in its entirety).

Various mutations in the C_H2-C_H3 region of IgG that can increase the affinity of Fc region to FcRn at an acidic pH are known in the art. These include, but are not limited to, modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at 428 and/or 433 (e.g., L/R/S/P/Q or K) and/or 434 (e.g., H/F or Y); or a modification at 250 and/or 428; or a modification at 307 or 308 (e.g., 308F, V308F), and 434. In another example, the modification can comprise a 428L (e.g., M428L) and 434S (e.g., N434S) modification; a 428L, 259I (e.g., V259I), and 308F (e.g., V308F) modification; a 433K (e.g., H433K) and a 434 (e.g., 434Y) modification; a 252, 254, and 256 (e.g., 52Y, 254T, and 256E) modification; a 250Q and 428L modification, or a combination thereof.

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 252 and 257, wherein the modification increases the affinity of the human C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H2 amino acid sequence comprising at least one modification between amino acid residues at positions 307 and 311, wherein the modification increases the affinity of the C_H2 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the heavy chain constant region nucleotide sequence encodes a human C_H3 amino acid sequence, wherein the C_H3 amino acid sequence comprises at least one modification between amino acid residues at positions 433 and 436, wherein the modification increases the affinity of the C_H3 amino acid sequence to FcRn in an acidic environment (e.g., in an endosome where pH ranges from about 5.5 to about 6.0).

In one embodiment, the human constant region amino acid sequence encoded by the heavy chain constant region nucleotide sequence described herein comprises a mutation selected from the group consisting of M428L, N434S, and a combination thereof. In one embodiment, the human constant region amino acid sequence comprises a mutation selected from the group consisting of M428L, V259I, V308F, and a combination thereof. In one embodiment, the human constant region amino acid sequence comprises an N434A mutation.

In one embodiment, the human constant region amino acid sequence comprises a mutation selected from the group consisting of M252Y, S254T, T256E, and a combination thereof. In one embodiment, the human constant region amino acid sequence comprises a mutation selected from the group consisting of T250Q, M248L, or both. In one embodiment, the human constant region amino acid sequence comprises a mutation selected from the group consisting of H433K, N434Y, or both.

In one embodiment, the heavy chain constant region amino acid sequence is a non-human constant region amino acid sequence, and the heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

In one embodiment, the heavy chain constant region nucleotide sequence is a human heavy chain constant region amino acid sequence, and the human heavy chain constant region amino acid sequence comprises one or more of any of the types of modifications described above.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1

Construction of Humanized Immunoglobulin Heavy Chain Loci Comprising Histidine-Substituted D Gene Segments

Construction of immunoglobulin heavy chain loci comprising histidine-substituted human D gene segments was carried out by series of homologous recombination reactions in bacterial cells (BHR) using Bacterial Artificial Chromosome (BAC) DNA. Several targeting constructs for creation of a genetically engineered mouse that expresses a heavy chain variable domain comprising one or more histidine residues were generated using VELOCIGENE® genetic engineering technology (see, e.g., U.S. Pat. No. 6,586,251 and Valenzuela, D. M. et al. (2003), High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, *Nature Biotechnology* 21(6):652-659, which is incorporated herein by reference in their entireties).

Initially, human D gene segments were synthesized in silico as four pieces (4 repeats) in which the codons encoding tyrosine (Y), asparagine (N), serine (S), glycine (G), and aspartate (D) in the hydrophilic frame were substituted with histidine codons (hereinafter "histidine-substituted human D gene segments", i.e., HD 1.1-6.6 (9586 bp; SEQ ID NO: 1), HD 1.7-6.13 (9268 bp; SEQ ID NO: 2), HD 1.14-6.19 (9441 bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592 bp; SEQ ID NO: 4) (FIG. **3**). The four repeats also contained unique restriction enzyme sites at the ends for ligating them back together. The specific location of the histidine substitutions (labeled in bold type) in each human D gene segment is shown in FIGS. **1**A and **1**B in the column labeled "Hydrophilic." As shown in FIG. **1**, while the modification introduced histidine codons in the hydrophilic reading frame, it also changed some stop codons to serine codons in the "Stop" reading frame. The modification, however, made few changes in the "Hydrophobic" reading frame. The detailed procedure for ligating the four synthesized D segment repeats is illustrated in FIG. **3** (sequential ligation). The resulting clone contained, from 5' to 3', a 5' mouse homology arm, a floxed neomycin cassette, human D gene segments comprising histidine substitutions (i.e., HD 1.1-6.6 (9586 bp; SEQ ID NO: 1), HD 1.7-6.13 (9268 bp; SEQ ID NO: 2), HD 1.14-6.19 (9441 bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592 bp; SEQ ID NO: 4)), a chloramphenicol selection cassette, and a 3' homology arm.

The following six genetic modifications were carried out in order to replace the endogenous human D gene segments in the VELOCIMMUNE® humanized mouse with the histidine-substituted human D gene segments described above.

First, pLMa0174, containing a spectinomycin selection cassette and an AsiSI restriction site, was targeted into the 5' end of the MAID 1116 clone (Step 1. BHR (Spec); FIG. **2**). During Step 1, a chloramphenicol selection cassette, a neomycin selection cassette, a loxP site, two V_H gene segments (hV_H1-3 and hV_H1-2), and the human Adam6p gene, all of which are located 5' upstream of hV_H6-1, were deleted from the MAID 1116 clone and replaced by a spectinomycin cassette to yield the V1433 clone.

Second, in Step 2 (BHR (Hyg+Spec); FIG. **2**), pNTu0002 containing a hygromycin cassette flanked by FRT sites was targeted into a region comprising human immunoglobulin D_H gene segments. During Step 2, all human heavy chain D gene segments were deleted from V1433 and replaced with the hygromycin cassette to yield MAID6011 VI 434 (clone 1). The modification also introduced the PI-SceI and the I-CeuI restriction sites at the 5' and 3' end of the hygromycin cassette.

Third, the genomic region comprising histidine-substituted human D gene segments (HD 1.1-6.6 (9586 bp; SEQ ID NO: 1), HD 1.7-6.13 (9268 bp; SEQ ID NO: 2), HD 1.14-6.19 (9441 bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592 bp; SEQ ID NO: 4)) were introduced into a region between the PI-SceI and the I-CeuI sites of MAID 6011 V1434 via restriction digestion and ligation (PI-SceI/I-CeuI Ligation modified 1116 (Kan+Spec); FIG. **4**). This yielded MAID6012 V1469 containing, from 5' to 3', a spectinomycin cassette, about 50 kb of a genomic region comprising V_H6-1, a floxed neomycin cassette, about 40 kb of the histidine-substituted human D gene segments (HD 1.1-6.6 (9586 bp; SEQ ID NO: 1), HD 1.7-6.13 (9268 bp; SEQ ID NO: 2), HD 1.14-6.19 (9441 bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592 bp; SEQ ID NO: 4)), and about 25 kb of a genomic region containing human J_H gene segments, followed by a mouse E_i(mIgH intronic enhancer; SEQ ID NO: 5), a mouse switch region (SEQ ID NO: 6), and a mouse IgM constant region nucleotide sequence (mIgM exon 1; SEQ ID NO: 7). Bacterial cells containing the modification were selected based on Kanamycin and Spectinomycin selection.

Fourth, MAID 1460 heterozygous mouse ES cells were targeted with MAID 6011 V1434 via electroporation in order to remove all endogenous human D gene segments from the MAID 1460 clone as illustrated in FIG. **5**. This yielded MAID 6011 heterozygous mouse ES cells comprising in its immunoglobulin heavy chain locus (at the 129 strain-derived chromosome), from 5' to 3', an FRT site, human V_H gene segments, a mouse genomic region encompassing adam6a/b genes, a hygromycin cassette flanked by FRT sites, and human J_H segments, followed by a mouse E_i sequence and an IgM constant region nucleotide sequence. The genetic modi-

fication of MAID 6011 (a loss of alleles, a gain of alleles, and presence of parental alleles) was confirmed by using the probes and primers as shown in FIG. **6**.

Fifth, MAID 6011 heterozygous mouse ES cells were electroporated with MAID 6012 V1469 in order to introduce histidine-substituted human D gene segments (i.e., HD 1.1-6.6 (9586 bp; SEQ ID NO: 1), HD 1.7-6.13 (9268 bp; SEQ ID NO: 2), HD 1.14-6.19 (9441 bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592 bp; SEQ ID NO: 4)) into MAID 6011. The targeting step removed the floxed hygromycin selection cassette from MAID 6011 and replaced the sequence with the histidine-substituted human D gene segments. This lead to MAID 6012 hetrozygous ES cells comprising a wild-type C57BL/6 strain-derived chromosome and a genetically modified 129 strain-derived chromosome comprising human wild-type V_H and J_H gene segments and the histidine-substituted human D gene segments described herein. In addition, the ES cells contained a mouse genomic region encompassing adam6a/b genes and a floxed neomycin cassette between the V_H and D segments (FIG. **7**). The genetic modification of MAID 6012 (a loss of alleles, a gain of alleles, and presence of parental alleles) was confirmed by using the probes and primers as shown in FIG. **8**.

Lastly, MAID 6012 ES cells were electroporated with a plasmid that expresses a Cre recombinase in order to remove the neomycin selection cassette from the MAID 6012 ES cells, resulting in MAID 6013 heterozygous ES cells (FIG. **9**). The final MAID 6013 heterozygous ("MAID 6013 het") ES cell contains a wild-type C57BL/6 strain-derived chromosome and a genetically modified, 129 strain-derived chromosome comprising in its immunoglobulin heavy chain locus, from 5' to 3', (1) an FRT site; (2) human V_H gene segments; (3) a mouse genomic region encompassing adam6a/b genes; (4) a floxed neomycin selection cassette; (5) histidine-substituted human D gene segments (HD 1.1-6.6 (9586 bp; SEQ ID NO: 1), HD 1.7-6.13 (9268 bp; SEQ ID NO: 2), HD 1.14-6.19 (9441 bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592 bp; SEQ ID NO: 4)); (6) human J_H gene segments; followed by (7) a mouse E, sequence (mIgH intronic enhancer; SEQ ID NO: 5), (8) a switch region (SEQ ID NO: 6); and (9) a mouse IgM constant region nucleotide sequence (mIgM exon 1; SEQ ID NO: 7) as illustrated in FIG. **9**.

The targeted ES cells (MAID 6013) described above were used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® method (see, e.g., U.S. Pat. No. 7,576,259, U.S. Pat. No. 7,659,442, U.S. Pat. No. 7,294,754, US 2008-0078000 A1, all of which are incorporated by reference herein in their entireties). Mice bearing the genetically modified immunoglobulin heavy chain locus comprising the histidine-substituted human heavy chain D gene segments described herein were identified by genotyping using the primers and probes set forth in FIG. **8**. The resulting genetically modified F0 mouse was crossed to a wild-type mouse to obtain F1 offspring. F1 pups were genotyped, and the F1 pups that are heterozygous for the genetically modified immunoglobulin locus comprising histidine-substituted human heavy chain D gene segments were selected for further characterization.

Example 2

Analysis of Rearranged Heavy Chain Variable Region Nucleotide Sequences

Next, it was examined whether the genetically modified mouse comprising histidine-substituted human D gene segments described herein, i.e., 6013 F0 heterozygous mouse, which comprises in its germline a 129 strain-derived chromosome comprising human V_H, J_H gene segments, and histidine-substituted human D gene segments (HD 1.1-6.6 (9586 bp; SEQ ID NO: 1), HD 1.7-6.13 (9268 bp; SEQ ID NO: 2), HD 1.14-6.19 (9441 bp; SEQ ID NO: 3), and HD 1.20-6.25, 1.26 (11592 bp; SEQ ID NO: 4), can express rearranged heavy chain V(D)J sequences comprising one or more histidine codons derived from the genetically modified immunoglobulin heavy chain locus.

To this end, mRNA sequences encoding IgM heavy chain variable region were analyzed for the presence of IgM CDR3 sequences derived from the histidine-substituted human D gene segments via high throughput sequencing. Briefly, spleens were harvested and homogenized in 1×PBS (Gibco) using glass slides. Cells were pelleted in a centrifuge (500×g for 5 minutes), and red blood cells were lysed in ACK Lysis buffer (Gibco) for 3 minutes. Cells were washed with 1×PBS and filtered using a 0.7 μm cell strainer. B-cells were isolated from spleen cells using MACS magnetic positive selection for CD19 (Miltenyi Biotec). Total RNA was isolated from pelleted B-cells using the RNeasy Plus kit (Qiagen). PolyA+ mRNA was isolated from total RNA using the Oligotex® Direct mRNA mini kit (Qiagen).

Double-stranded cDNA was prepared from splenic B cell mRNA by 5' RACE using the SMARTer™ Pico cDNA Synthesis Kit (Clontech). The Clontech reverse transcriptase and dNTPs were substituted with Superscript II and dNTPs from Invitrogen. Heavy chain variable region (V_H) antibody repertoires were amplified from the cDNA using primers specific for IgM constant regions and the SMARTer™ 5' RACE primer (Table 1). PCR products were cleaned up using a QIAquick® PCR Purification Kit (Qiagen). A second round of PCR was done using the same 5' RACE primer and a nested 3' primer specific for the IgM constant regions (Table 2). The second round PCR products were purified using a SizeSelect™ E-Gel® system (Invitrogen). A third PCR was performed with primers that added 454 adapters and barcodes. The third round PCR products were purified using Agencourt® AMPure® XP Beads. Purified PCR products were quantified by SYBR®-qPCR using a KAPA Library Quantification Kit (KAPA Biosystems). Pooled libraries were subjected to emulsion PCR (emPCR) using the 454 GS Junior Titanium Series Lib-A emPCR Kit (Roche Diagnostics) and bidirectional sequencing using Roche 454 GS Junior instrument according to the manufacturers protocols.

TABLE 1

NAME	SEQUENCE
3' mIgM CH1 outer	TCTTATCAGACAGGGGGCTCTC (SEQ ID NO: 321)

TABLE 2

NAME	SEQUENCE
3' mIgM CH1 inner	GGAAGACATTTGGGAAGGACTG (SEQ ID NO: 322)

Bioinfomatic Analysis

The 454 sequences were sorted based on the sample barcode perfect match and trimmed for quality. Custom D database was created using histidine-substituted human D-gene segments. Sequences were annotated based on alignment of rearranged Ig sequences to human germline V and J gene segments database using local installation of igblast (NCBI,

v2.2.25+). Sequences derived from the endogenous mouse immunoglobulin heavy chain locus were filtered out using similarity threshold of 90%. A sequence was marked as ambiguous and removed from analysis when multiple best hits with identical score were detected. A set of perl scripts was developed to analyze results and store data in mysql database. The CDR3 region was defined between conserved C codon and FGXG motif (SEQ ID NO: 323) for light chains and WGXG motif (SEQ ID NO: 324) for heavy chains. CDR3 length was determined using only productive antibodies. Number of histidine codons was calculated for each CDR3 region.

As shown in FIGS. **11-13**, the 6013 F0 heterozygous mice expressed a diverse repertoire of rearranged heavy chain variable region mRNA sequences (rearranged V-D-J sequences) encoding one or more histidine codons in CDR3. The sequencing and alignment data suggested that the histidine codons appeared in CDR3 sequences were derived from various histidine-substituted human D gene segments present in the genetically modified immunoglobulin heavy chain locus of the 6013 mice described herein. In addition, as compared with control mice comprising human V_H, D_H, J_H gene segments and mouse adam6 genes (VI3-Adam6, US Publication No. 2012/0322108A1, which is incorporated by reference in its entirety), the genetically modified 6013 F0 heterozygous mice exhibited a higher frequency of histidine occurrence in the heavy chain CDR3 sequences (FIG. **14**).

While the described invention has been described with reference to the specific embodiments thereof it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adopt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the described invention. All such modifications are intended to be within the scope of the claims appended hereto.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 324

<210> SEQ ID NO 1
<211> LENGTH: 9586
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(9586)
<223> OTHER INFORMATION: HD 1.1-6.6

<400> SEQUENCE: 1

tccccgttga agctgacctg cccagagggg cctgggccca ccccacacac cggggcggaa 60

tgtgtacagg ccccggtctc tgtgggtgtt ccgctaactg gggctcccag tgctcacccc 120

acaactaaag cgagccccag cctccagagc ccccgaagga gatgccgccc acaagcccag 180

cccccatcca ggaggcccca gagctcaggg cgccggggca gattctgaac agccccgagt 240

cacggtgggt accactggca cgaccaccgt gagaaaaact gtgtccaaaa ctgtctcctg 300

gcccctgctg gaggccgcgc cagagagggg agcagccgcc ccgaacctag gtcctgctca 360

gctcacacga cccccagcac ccagagcaca acggagtccc cattgaatgg tgaggacggg 420

gaccagggct ccaggggtc atggaagggg ctggacccca tcctactgct atggtcccag 480

tgctcctggc cagaactgac cctaccaccg acaagagtcc ctcagggaaa cgggggtcac 540

tggcacctcc cagcatcaac cccaggcagc acaggcataa accccacatc cagagccgac 600

tccaggagca gagacacccc agtaccctgg gggacaccga ccctgatgac tccccactgg 660

aatccacccc agagtccacc aggaccaaag accccgcccc tgtctctgtc cctcactcag 720

gacctgctgc ggggcgggcc atgagaccag actcgggctt agggaacacc actgtggccc 780

caacctcgac caggccacag gcccttcctt cctgccctgc ggcagcacag actttggggt 840

ctgtgcagag aggaatcaca gaggccccag gctgaggtgg tgggggtgga agacccccag 900

gaggtggccc acttcccttc ctcccagctg gaacccacca tgaccttctt aagatagggg 960

tgtcatccga ggcaggtcct ccatggagct cccttcaggc tcctccccgg tcctcactag 1020

gcctcagtcc cggctgcggg aatgcagcca ccacaggcac accaggcagc ccagacccag 1080

ccagcctgca gtgcccaagc ccacattctg gagcagagca ggctgtgtct gggagagtct 1140

gggctcccca ccgccccccc gcacacccca cccacccctg tccaggccct atgcaggagg 1200

gtcagagccc cccatggggt atggacttag ggtctcactc acgtggctcc cctcctgggt 1260

-continued

gaaggggtct catgcccaga tccccacagc agagctggtc aaaggtggag gcagtggccc 1320

cagggccacc ctgacctgga ccctcaggct cctctagccc tggctgccct gctgtccctg 1380

ggaggcctgg actccaccag accacaggtc cagggcaccg cccataggtg ctgcccacac 1440

tcagttcaca ggaagaagat aagctccaga cccccaagac tgggacctgc cttcctgcca 1500

ccgcttgtag ctccagacct ccgtgcctcc cccgaccact tacacacggg ccagggagct 1560

gttccacaaa gatcaacccc aaaccgggac cgcctggcac tcgggccgct gccacttccc 1620

tctccatttg ttcccagcac ctctgtgctc cctccctcct ccctccttca ggggaacagc 1680

ctgtgcagcc cctccctgca ccccacaccc tggggaggcc caaccctgcc tccagccctt 1740

tctcccccgc tgctcttcct gcccatccag acaaccctgg ggtcccatcc ctgcagccta 1800

caccctggtc tccacccaga cccctgtctc tccctccaga cacccctccc aggccaaccc 1860

tgcacatgca ggccctcccc ttttctgctg ccagagcctc agtttctacc ctctgtgcct 1920

acccccctgcc tcctcctgcc cacaactcga gctcttcctc tcctggggcc cctgagccat 1980

ggcactgacc gtgcactccc acccccacac tgcccatgcc ctcaccttcc tcctggacac 2040

tctgaccccg ctcccctctt ggacccagcc ctggtatttc caggacaaag gctcacccaa 2100

gtcttcccca tgcaggccct tgccctcact gcccggttac acggcagcct cctgtgcaca 2160

gaagcaggga gctcagccct tccacaggca gaaggcactg aaagaaatcg gcctccagca 2220

ccctgatgca cgtccgcctg tgtctctcac tgcccgcacc tgcagggagg ctcggcactc 2280

cctgtaaaga cgagggatcc aggcagcaac atcatgggag aatgcagggc tcccagacag 2340

cccagccctc tcgcaggcct ctcctgggaa gagacctgca gccaccactg aacagccacg 2400

gagcccgctg gatagtaact gagtcagtga ccgacctgga gggcagggga gcagtgaacc 2460

ggagcccaga ccatagggac agagaccagc cgctgacatc ccgagcccct cactggcggc 2520

cccagaacac cgcgtggaaa cagaacagac ccacattccc acctggaaca gggcagacac 2580

tgctgagccc ccagcaccag ccctgagaaa caccaggcaa cggcatcaga gggggctcct 2640

gagaaagaaa ggaggggagg tctccttcac cagcaagtac ttcccttgac caaaaacagg 2700

gtccacgcaa ctccccccagg acaaaggagg agccccctgt acagcactgg gctcagagtc 2760

ctctcccaca caccctgagt ttcagacaaa aacccccctgg aaatcatagt atcagcagga 2820

gaactagcca gagacagcaa gaggggactc agtgactccc gcggggacag gaggattttg 2880

tgggggctcg tgtcactgtg aggacattgt agtcatacca gctgccatac ccacagtgac 2940

acagccccat tcccaaagcc ctgctgtaaa cgcttccact tctggagctg aggggctggg 3000

gggagcgtct gggaagtagg gcctaggggt ggccatcaat gcccaaaacg caccagactc 3060

ccccccagac atcaccccac tggccagtga gcagagtaaa cagaaaatga gaagcagctg 3120

ggaagcttgc acaggcccca aggaaagagc tttggcgggt gtgcaagagg ggatgcgggc 3180

agagcctgag cagggccttt tgctgtttct gctttcctgt gcagatagtt ccataaactg 3240

gtgttcaaga tcgatggctg ggagtgagcc caggaggaca gtgtgggaag ggcacaggga 3300

aggagaagca gccgctatcc tacactgtca tctttcaaga gtttgccctg tgcccacaat 3360

gctgcatcat gggatgctta acagctgatg tagacacagc taaagagaga atcagtgaaa 3420

tggatttgca gcacagatct gaataaattc tccagaatgt ggagccacac agaagcaagc 3480

acaaggaaag tgcctgatgc aagggcaaag tacagtgtgt accttcaggc tgggcacaga 3540

cactctgaaa agccttggca ggaactccct gcaacaaagc agagccctgc aggcaatgcc 3600

-continued

agctccagag ccctccctga gagcctcatg ggcaaagatg tgcacaacag gtgtttctca 3660

tagccccaaa ctgagaatga agcaaacagc catctgaagg aaaacaggca aataaacgat 3720

ggcaggttca tgaaatgcaa acccagacag ccagaaggac aacagtgagg gttacaggtg 3780

actctgtggt tgagttcatg acaatgctga gtaattggag taacaaagga aagtccaaaa 3840

aatactttca atgtgatttc ttctaaataa aatttacagc cggcaaaatg aactatcttc 3900

ttaagggata aactttccac taggaaaact ataaggaaaa tcaagaaaag gatgatcaca 3960

taaacacagt ggtcgttact tctactgggg aaggaagagg gtatgaactg agacacacag 4020

ggttggcaag tctcctaaca agaacagaac aaatacatta cagtaccttg aaaacagcag 4080

ttaaaattct aaattgcaag aagaggaaaa tgcacacagc tgtgtttaga aaattctcag 4140

tccagcactg ttcataatag caaagacatt aacccaggtt ggataaataa acgatgacac 4200

aggcaattgc acaatgatac agacatacat tcagtatatg agacattgat gatgtatccc 4260

caaagaaatg actttaaaga gaaaaggcct gatatgtggt ggcactcacc tccctgggca 4320

tccccggaca ggctgcaggc acactgtgtg gcagggcagg ctggtacctg ctggcagctc 4380

ctggggcctg atgtggagca ggcacagagc cgtatccccc cgaggacata tacccccaag 4440

gacggcacag ttggtacatt ccggagacaa gcaactcagc cacactccca ggccagagcc 4500

cgagagggac gcccatgcac agggaggcag agcccagctc ctccacagcc agcagcaccc 4560

gtgcaggggc cgccatctgg caggcacaga gcatgggctg ggaggagggg cagggacacc 4620

aggcagggtt ggcaccaact gaaaattaca gaagtctcat acatctacct cagccttgcc 4680

tgacctgggc ctcacctgac ctggacctca cctggcctgg acctcacctg gcctagacct 4740

cacctctggg cttcacctga gctcggcctc acctgacttg gaccttgcct gtcctgagct 4800

cacatgatct gggcctcacc tgacctgggt ttcacctgac ctgggcttca cctgacctgg 4860

gcctcatctg acctgggcct cactggcctg gacctcacct ggcctgggct tcacctggcc 4920

tcaggcctca tctgcacctg ctccaggtct tgctggaacc tcagtagcac tgaggctgca 4980

ggggctcatc cagggttgca gaatgactct agaacctccc acatctcagc tttctgggtg 5040

gaggcacctg gtggcccagg gaatataaaa agcctgaatg atgcctgcgt gatttggggg 5100

caatttataa acccaaaagg acatggccat gcagcgggta gggacaatac agacagatat 5160

cagcctgaaa tggagcctca gggcacaggt gggcacggac actgtccacc taagccaggg 5220

gcagacccga gtgtccccgc agtagacctg agagcgctgg gcccacagcc tcccctcggt 5280

gccctgctac ctcctcaggt cagccctgga catcccgggt ttccccaggc ctggcggtag 5340

gtttggggtg aggtctgtgt cactgtggta tcaccatttt tggagtggtc attataccca 5400

cagtgtcaca gagtccatca aaaacccatc cctgggaacc ttctgccaca gccctccctg 5460

tggggcaccg ccgcgtgcca tgttaggatt ttgactgagg acacagcacc atgggtatgg 5520

tggctaccgc agcagtgcag cccgtgaccc aaacacacag ggcagcaggc acaacagaca 5580

agcccacaag tgaccaccct gagctcctgc ctgccagccc tggagaccat gaaacagatg 5640

gccaggatta tcccataggt cagccagacc tcagtccaac aggtctgcat cgctgctgcc 5700

ctccaatacc agtccggatg gggacagggc tggcccacat taccatttgc tgccatccgg 5760

ccaacagtcc cagaagcccc tccctcaagg ctgggccaca tgtgtggacc ctgagagccc 5820

cccatgtctg agtaggggca ccaggaaggt ggggctggcc ctgtgcactg tccctgcccc 5880

tgtggtccct ggcctgcctg gccctgacac ctgggcctct cctgggtcat ttccaagaca 5940

gaagacattc ccaggacagc tggagctggg agtccatcat cctgcctggc cgtcctgagt 6000

-continued

cctgcgcctt tccaaacctc acccgggaag ccaacagagg aatcacctcc cacaggcaga 6060

gacaaagacc ttccagaaat ctctgtctct ctccccagtg ggcaccctct tccagggcag 6120

tcctcagtga tatcacagtg ggaacccaca tctggatcgg gactgccccc agaacacaag 6180

atggcccaca gggacagccc cacagcccag cccttcccag acccctaaaa ggcgtcccac 6240

cccctgcatc tgccccaggg ctcaaactcc aggaggactg actcctgcac accctcctgc 6300

cagacatcac ctcagcccct cctggaaggg acaggagcgc gcaagggtga gtcagaccct 6360

cctgccctcg atggcaggcg gagaagattc agaaaggtct gagatcccca ggacgcagca 6420

ccactgtcaa tgggggcccc agacgcctgg accagggcct gcgtgggaaa ggcctctggg 6480

cacactcagg ggctttttgt gaagggtcct cctactgtgt gaccacagtc actaccacag 6540

tgatgaaccc agcagcaaaa actgaccgga ctcccaaggt ttatgcacac ttctccgctc 6600

agagctctcc aggatcagaa gagccgggcc caagggtttc tgcccagacc ctcggcctct 6660

agggacatct tggccatgac agcccatggg ctggtgcccc acacatcgtc tgccttcaaa 6720

caagggcttc agagggctct gaggtgacct cactgatgac cacaggtgcc ctggcccctt 6780

ccccaccagc tgcaccagac cccgtcatga cagatgcccc gattccaaca gccaattcct 6840

ggggccagga atcgctgtag acaccagcct ccttccaaca cctcctgcca attgcctgga 6900

ttcccatccc ggttggaatc aagaggacag catcccccag gctcccaaca ggcaggactc 6960

ccacaccctc ctctgagagg ccgctgtgtt ccgtagggcc aggctgcaga cagtcccccc 7020

cacctgccac tagacaaatg cctgctgtag atgtccccac ctggaaaata ccactcatgg 7080

agcccccagc cccaggtaca gctgtagaga gagtctctga ggcccctaag aagtagccat 7140

gcccagttct gccgggaccc tcggccaggc tgacaggagt ggacgctgga gctgggccca 7200

tactgggcca cataggagct caccagtgag ggcaggagag cacatgccgg ggagcaccca 7260

gcctcctgct gaccagaggc ccgtcccaga gcccaggagg ctgcagaggc ctctccaggg 7320

ggacactgtg catgtctggt ccctgagcag ccccccacgt ccccagtcct gggggccccc 7380

ggcacagctg tctggaccct ctctattccc tgggaagctc ctcctgacag ccccgcctcc 7440

agttccaggt gtggttattg tcaggggggtg tcagactgtg gtggacacag ccatggttac 7500

cacagtggtg ctgcccatag cagcaaccag gccaagtaga caggcccctg ctgtgcagcc 7560

ccaggcctcc agctcacctg cttctcctgg ggctctcaag gctgctgttt tctgcactct 7620

cccctctgtg gggagggttc cctcagtggg agatctgttc tcaacatccc acggcctcat 7680

tcctgcaagg aaggccaatg gatgggcaac ctcacatgcc gcggctaaga tagggtgggc 7740

agcctggcgg ggacaggaca tcctgctggg gtatctgtca ctgtgcctag tggggcactg 7800

gctcccaaac aacgcagtcc ttgccaaaat ccccacggcc tcccccgcta ggggctggcc 7860

tgatctcctg cagtcctagg aggctgctga cctccagaat ggctccgtcc ccagttccag 7920

ggcgagagca gatcccaggc cggctgcaga ctgggaggcc acccccctcct tcccagggtt 7980

cactgcaggt gaccagggca ggaaatggcc tgaacacagg gataaccggg ccatccccca 8040

acagagtcca ccccctcctg ctctgtaccc cgcacccccc aggccagccc atgacatccg 8100

acaaccccac accagagtca ctgcccggtg ctgccctagg gaggacccct cagcccccac 8160

cctgtctaga ggactgggga ggacaggaca cgccctctcc ttatggttcc cccacctggc 8220

tctggctggg acccttgggg tgtggacaga aaggacgctt gcctgattgg cccccaggag 8280

cccagaactt ctctccaggg accccagccc gagcaccccc ttacccagga cccagccctg 8340

-continued

```
cccctcctcc cctctgctct cctctcatca ccccatggga atccagaatc cccaggaagc   8400

catcaggaag ggctgaggga ggaagtgggg ccactgcacc accaggcagg aggctctgtc   8460

tttgtgaacc cagggaggtg ccagcctcct agagggtatg gtccaccctg cctatggctc   8520

ccacagtggc aggctgcagg gaaggaccag ggacggtgtg ggggagggct cagggccccg   8580

cgggtgctcc atcttggatg agcctatctc tctcacccac ggactcgccc acctcctctt   8640

caccctggcc acacgtcgtc cacaccatcc taagtcccac ctacaccaga gccggcacag   8700

ccagtgcaga cagaggctgg ggtgcagggg ggccgactgg gcagcttcgg ggagggagga   8760

atggaggaag gggagttcag tgaagaggcc cccctcccct gggtccagga tcctcctctg   8820

ggaccccccgg atcccatccc ctccaggctc tgggaggaga agcaggatgg gagaatctgt   8880

gcgggaccct ctcacagtgg aatacctcca cagcggctca ggccagatac aaaagcccct   8940

cagtgagccc tccactgcag tgctgggcct gggggcagcc gctcccacac aggatgaacc   9000

cagcaccccg aggatgtcct gccaggggga gctcagagcc atgaaggagc aggatatggg   9060

acccccgata caggcacaga cctcagctcc attcaggact gccacgtcct gccctgggag   9120

gaaccccttt ctctagtccc tgcaggccag gaggcagctg actcctgact tggacgccta   9180

ttccagacac cagacagagg ggcaggcccc ccagaaccag ggatgaggac gccccgtcaa   9240

ggccagaaaa gaccaagttg cgctgagccc agcaagggaa ggtccccaaa caaaccagga   9300

agtttctgaa ggtgtctgtg tcacagtgga gcatagccac tcgtcccaca gtgacactcg   9360

ccaggccaga aaccccatcc caagtcagcg gaatgcagag agagcaggga ggacatgttt   9420

aggatctgag gccgcacctg acacccaggc cagcagacgt ctcctgtcca cggcaccctg   9480

ccatgtcctg catttctgga agaacaaggg caggctgaag ggggtccagg accaggagat   9540

gggtccgctc tacccagaga aggagccagg caggacacaa gccccc                  9586


<210> SEQ ID NO 2
<211> LENGTH: 9268
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(9268)
<223> OTHER INFORMATION: HD 1.7-6.13

<400> SEQUENCE: 2

tccccattga ggctgacctg cccagagggt cctgggccca cccaacacac cggggcggaa     60

tgtgtgcagg cctcggtctc tgtgggtgtt ccgctagctg gggctcacag tgctcacccc    120

acacctaaaa cgagccacag cctccggagc ccctgaagga gaccccgccc acaagcccag    180

cccccaccca ggaggcccca gagcacaggg cgccccgtcg gattctgaac agccccgagt    240

cacagtgggt atcactggca ctaccactgt gagaaaagct tcgtccaaaa cggtctcctg    300

gccacagtcg gaggccccgc cagagagggg agcagccacc ccaaacccat gttctgccgg    360

ctcccatgac cccgtgcacc tggagcccca cggtgtcccc actggatggg aggacaaggg    420

ccgggggctc cggcgggtcg gggcaggggc ttgatggctt ccttctgccg tggccccatt    480

gcccctggct ggagttgacc cttctgacaa gtgtcctcag agagtcaggg atcagtggca    540

cctcccaaca tcaaccccac gcagcccagg cacaaacccc acatccaggg ccaactccag    600

gaacagagac accccaatac cctgggggac cccgaccctg atgactcccg tcccatctct    660

gtccctcact tggggcctgc tgcggggcga gcacttggga gcaaactcag gcttagggga    720

caccactgtg ggcctgacct cgagcaggcc acagaccctt ccctcctgcc ctggtgcagc    780
```

-continued

acagactttg gggtctgggc agggaggaac ttctggcagg tcaccaagca cagagccccc 840

aggctgaggt ggccccaggg ggaaccccag caggtggccc actacccttc ctcccagctg 900

gaccccatgt cttccccaag ataggggtgc catccaaggc aggtcctcca tggagccccc 960

ttcaggctcc tctccagacc ccactgggcc tcagtcccca ctctaggaat gcagccacca 1020

cgggcacacc aggcagccca ggcccagcca ccctgcagtg cccaagccca caccctggag 1080

gagagcaggg tgcgtctggg aggggctggg ctccccaccc ccacccccac ctgcacaccc 1140

cacccaccct tgcccgggcc ccctgcagga gggtcagagc ccccatggga tatggactta 1200

gggtctcact cacgcacctc ccctcctggg agaaggggtc tcatgcccag atccccccag 1260

cagcgctggt cacaggtaga ggcagtggcc ccagggccac cctgacctgg cccctcaggc 1320

tcctctagcc ctggctgccc tgctgtccct gggaggcctg ggctccacca gaccacaggt 1380

ctagggcacc gcccacactg gggccgccca cacacagctc acaggaagaa gataagctcc 1440

agacccccag gcccgggacc tgccttgctg ctacgacttc ctgccccaga cctcgttgcc 1500

ctcccccgtc cacttacaca caggccagga agctgttccc acacagacca accccagacg 1560

gggaccacct ggcactcagg tcactgccat ttccttctcc attcacttcc aatgcctctg 1620

tgcttcctcc ctcctccttc cttcgggggа gcaccctgtg cagctcctcc ctgcagtcca 1680

caccctgggg agacccgacc ctgcagccca caccctgggg agacctgacc ctcctccagc 1740

cctttctccc ccgctgctct tgccacccac caagacagcc ctggggtcct gtccctacag 1800

cccccaccca gttctctacc tagacccgtc ttcctccctc taaacacctc tcccaggcca 1860

accctacacc tgcaggccct cccctccact gccaaagacc ctcagtttct cctgcctgtg 1920

cccacccccg tgctcctcct gcccacagct cgagctcttc ctctcctagg gcccctgagg 1980

gatggcattg accgtgccct cgcacccaca cactgcccat gccctcacat tcctcctggc 2040

cactccagcc ccactccccт ctcaggcctg gctctggtat ttctgggaca aagccttacc 2100

caagtctttc ccatgcaggc ctgggcccтt accctcactg cccggttaca gggcagcctc 2160

ctgtgcacag aagcagggag ctcagcccтt ccacaggcag aaggcactga aagaaatcgg 2220

cctccagcgc cttgacacac gtctgcctgt gtctctcact gcccgcacct gcagggaggc 2280

tcggcactcc ctctaaagac gagggatcca ggcagcagca tcacaggaga atgcagggct 2340

accagacatc ccagtcctct cacaggcctc tcctgggaag agacctgaag acgcccagtc 2400

aacggagtct aacaccaaac ctccctggag gccgatgggt agtaacggag tcattgccag 2460

acctggaggc aggggagcag tgagcccgag cccacaccat agggccagag gacagccact 2520

gacatcccaa gccactcact ggtggtccca caacacccca tggaaagagg acagacccac 2580

agtcccacct ggaccagggc agagactgct gagacccagc accagaacca accaagaaac 2640

accaggcaac agcatcagag ggggctctgg cagaacagag gaggggaggt ctccttcacc 2700

agcaggcgct tcccttgacc gaagacagga tccatgcaac tcccccagga caaaggagga 2760

gccccttgtt cagcactggg ctcagagtcc tctccaagac acccagagtt tcagacaaaa 2820

acccccctgga atgcacagtc tcagcaggag agccagccag agccagcaag atggggctca 2880

gtgacacccg cagggacagg aggattttgt gggggctcgt gtcactgtga ggacattgta 2940

ctcatggtgt atgccatacc cacagtgaca cagccccatt cccaaagccc tactgcaaac 3000

gcattccact tctggggctg aggggctggg ggagcgtctg ggaaataggg ctcaggggtg 3060

tccatcaatg cccaaaacgc accagactcc cctccataca tcacacccac cagccagcga 3120

-continued

gcagagtaaa cagaaaatga gaagcaagct ggggaagctt gcacaggccc caaggaaaga 3180

gctttggcgg gtgtgtaaga ggggatgcgg gcagagcctg agcagggcct tttgctgttt 3240

ctgctttcct gtgcagagag ttccataaac tggtgttcga gatcaatggc tgggagtgag 3300

cccaggagga cagcgtggga agagcacagg gaaggaggag cagccgctat cctacactgt 3360

catctttcga aagtttgcct tgtgcccaca ctgctgcatc atgggatgct taacagctga 3420

tgtagacaca gctaaagaga gaatcagtga gatggatttg cagcacagat ctgaataaat 3480

tctccagaat gtggagcagc acagaagcaa gcacacagaa agtgcctgat gcaaggacaa 3540

agttcagtgg gcaccttcag gcattgctgc tgggcacaga cactctgaaa agccctggca 3600

ggaactccct gtgacaaagc agaaccctca ggcaatgcca gccccagagc cctccctgag 3660

agcctcatgg gcaaagatgt gcacaacagg tgtttctcat agccccaaac tgagagcaaa 3720

gcaaacgtcc atctgaagga gaacaggcaa ataaacgatg gcaggttcat gaaatgcaaa 3780

cccagacagc cacaagcaca aaagtacagg gttataagcg actctggttg agttcatgac 3840

aatgctgagt aattggagta acaaagtaaa ctccaaaaaa tactttcaat gtgatttctt 3900

ctaaataaaa tttacaccct gcaaaatgaa ctgtcttctt aagggataca tttcccagtt 3960

agaaaaccat aaagaaaacc aagaaaagga tgatcacata aacacagtgg tggttacttc 4020

tgctggggaa ggaagagggt atgaactgag atacacaggg tgggcaagtc tcctaacaag 4080

aacagaacga atacattaca gtaccttgaa aacagcagtt aaacttctaa attgcaagaa 4140

gaggaaaatg cacacagttg tgtttagaaa attctcagtc cagcactgtt cataatagca 4200

aagacattaa cccaggtcgg ataaataagc gatgacacag gcaattgcac aatgatacag 4260

acatatattt agtatatgag acatcgatga tgtatcccca aataaacgac tttaaagaga 4320

taaagggctg atgtgtggtg gcattcacct ccctgggatc cccggacagg ttgcaggctc 4380

actgtgcagc agggcaggcg ggtacctgct ggcagttcct ggggcctgat gtggagcaag 4440

cgcagggcca tatatcccgg aggacggcac agtcagtgaa ttccagagag aagcaactca 4500

gccacactcc ccaggcagag cccgagaggg acgcccacgc acagggaggc agagcccagc 4560

acctccgcag ccagcaccac ctgcgcacgg gccaccacct tgcaggcaca gagtgggtgc 4620

tgagaggagg ggcagggaca ccaggcaggg tgagcaccca gagaaaactg cagacgcctc 4680

acacatccac ctcagcctcc cctgacctgg acctcactgg cctgggcctc acttaacctg 4740

ggcttcacct gaccttggcc tcacctgact tggacctcgc ctgtcccaag ctttacctga 4800

cctgggcctc aactcacctg aacgtctcct gacctgggtt taacctgtcc tggaactcac 4860

ctggccttgg cttcccctga cctggacctc atctggcctg ggcttcacct ggcctgggcc 4920

tcacctgacc tggacctcat ctggcctgga cctcacctgg cctggacttc acctggcctg 4980

ggcttcacct gacctggacc tcacctggcc tcgggcctca cctgcacctg ctccaggtct 5040

tgctggagcc tgagtagcac tgagggtgca gaagctcatc cagggttggg gaatgactct 5100

agaagtctcc cacatctgac ctttctgggt ggaggcagct ggtggccctg ggaatataaa 5160

aatctccaga atgatgactc tgtgatttgt gggcaactta tgaacccgaa aggacatggc 5220

catggggtgg gtagggacat agggacagat gccagcctga ggtggagcct caggacacag 5280

gtgggcacgg acactatcca cataagcgag ggatagaccc gagtgtcccc acagcagacc 5340

tgagagcgct gggcccacag cctcccctca gagccctgct gcctcctccg gtcagccctg 5400

gacatcccag gtttccccag gcctggcggt aggtttagaa tgaggtctgt gtcactgtgg 5460

tatcaccata ttttgactgg tcattataac cacagtgtca cagagtccat caaaaaccca 5520

-continued

tgcctggaag cttcccgcca cagccctccc catggggccc tgctgcctcc tcaggtcagc 5580

cccggacatc ccgggtttcc ccaggctggg cggtaggttt ggggtgaggt ctgtgtcact 5640

gtggtatcac catggttcgg ggagtcatta taaccacagt gtcacagagt ccatcaaaaa 5700

cccatccctg ggagcctccc gccacagccc tccctgcagg ggaccggtac gtgccatgtt 5760

aggattttga tcgaggagac agcaccatgg gtatggtggc taccacagca gtgcagcctg 5820

tgacccaaac ccgcagggca gcaggcacga tggacaggcc cgtgactgac cacgctgggc 5880

tccagcctgc cagccctgga gatcatgaaa cagatggcca aggtcaccct acaggtcatc 5940

cagatctggc tccgaggggt ctgcatcgct gctgccctcc caacgccagt ccaaatggga 6000

cagggacggc ctcacagcac catctgctgc catcaggcca gcgatcccag aagcccctcc 6060

ctcaaggctg ggcacatgtg tggacactga gagccctcat atctgagtag gggcaccagg 6120

agggaggggc tggccctgtg cactgtccct gcccctgtgg tccctggcct gcctggccct 6180

gacacctgag cctctcctgg gtcatttcca agacagaaga cattcctggg gacagccgga 6240

gctgggcgtc gctcatcctg cccggccgtc ctgagtcctg ctcatttcca gacctcaccg 6300

gggaagccaa cagaggactc gcctcccaca ttcagagaca aagaaccttc cagaaatccc 6360

tgcctctctc cccagtggac accctcttcc aggacagtcc tcagtggcat cacagcggcc 6420

tgagatcccc aggacgcagc accgctgtca ataggggccc caaatgcctg gaccagggcc 6480

tgcgtgggaa aggcctctgg ccacactcgg gctttttgtg aagggccctc ctgctgtgtg 6540

accacagtca ctaccatagt gatgaaccca gtggcaaaaa ctggctggaa acccaggggc 6600

tgtgtgcacg cctcagcttg gagctctcca ggagcacaag agccgggccc aaggatttgt 6660

gcccagaccc tcagcctcta gggacacctg ggtcatctca gcctgggctg gtgccctgca 6720

caccatcttc ctccaaatag gggcttcaga gggctctgag gtgacctcac tcatgaccac 6780

aggtgacctg gcccttccct gccagctata ccagaccctg tcttgacaga tgccccgatt 6840

ccaacagcca attcctggga ccctgaatag ctgtagacac cagcctcatt ccagtacctc 6900

ctgccaattg cctggattcc catcctggct ggaatcaaga aggcagcatc cgccaggctc 6960

ccaacaggca ggactcccgc acaccctcct ctgagaggcc gctgtgttcc gcagggccag 7020

gccctggaca gttcccctca cctgccacta gagaaacacc tgccattgtc gtccccacct 7080

ggaaaagacc actcgtggag cccccagccc caggtacagc tgtagagaca gtcctcgagg 7140

cccctaagaa ggagccatgc ccagttctgc cgggaccctc ggccaggccg acaggagtgg 7200

acgctggagc tgggcccaca ctgggccaca taggagctca ccagtgaggg caggagagca 7260

catgccgggg agcacccagc ctcctgctga ccagaggccc gtcccagagc ccaggaggct 7320

gcagaggcct ctccagggag acactgtgca tgtctggtac ctaagcagcc ccccacgtcc 7380

ccagtcctgg gggcccctgg ctcagctgtc tgggccctcc ctgctccctg ggaagctcct 7440

cctgacagcc ccgcctccag ttccaggtgt ggttattgtc aggcgatgtc agactgtggt 7500

ggacatagtg gccaccatta ccacagtggt gccgcccata gcagcaacca ggccaagtag 7560

acaggcccct gctgcgcagc cccaggcatc cacttcacct gcttctcctg gggctctcaa 7620

ggctgctgtc tgtcctctgg ccctctgtgg ggagggttcc ctcagtggga ggtctgtgct 7680

ccagggcagg gatgattgag atagaaatca aaggctggca gggaaaggca gcttcccgcc 7740

ctgagaggtg caggcagcac cacggagcca cggagtcaca gagccacgga gcccccattg 7800

tgggcatttg agagtgctgt gcccccggca ggcccagccc tgatggggaa gcctgtccca 7860

-continued

tcccacagcc cgggtcccac gggcagcggg cacagaagct gccaggttgt cctctatgat 7920

cctcatccct ccagcagcat cccctccaca gtggggaaac tgaggcttgg agcaccaccc 7980

ggccccctgg aaatgaggct gtgagcccag acagtgggcc cagagcactg tgagtacccc 8040

ggcagtacct ggctgcaggg atcagccaga gatgccaaac cctgagtgac cagcctacag 8100

gaggatccgg ccccacccag gccactcgat taatgctcaa ccccctgccc tggagacctc 8160

ttccagtacc accagcagct cagcttctca gggcctcatc cctgcaagga aggtcaaggg 8220

ctgggcctgc cagaaacaca gcaccctccc tagccctggc taagacaggg tgggcagacg 8280

gctgtggacg ggacatattg ctggggcatt tctcactgtc acttctgggt ggtagctctg 8340

acaaaaacgc agaccctgcc aaaatcccca ctgcctcccg ctaggggctg gcctggaatc 8400

ctgctgtcct aggaggctgc tgacctccag gatggctccg tccccagttc cagggcgaga 8460

gcagatccca ggcaggctgt aggctgggag gccacccctg cccttgccgg ggttgaatgc 8520

aggtgcccaa ggcaggaaat ggcatgagca cagggatgac cgggacatgc cccaccagag 8580

tgcgcccctt cctgctctgc accctgcacc ccccaggcca gcccacgacg tccaacaact 8640

gggcctgggt ggcagcccca cccagacagg acagacccag caccctgagg aggtcctgcc 8700

agggggagct aagagccatg aaggagcaag atatggggcc cccgatacag gcacagatgt 8760

cagctccatc caggaccacc cagcccacac cctgagagga acgtctgtct ccagcctctg 8820

caggtcggga ggcagctgac ccctgacttg gacccctatt ccagacacca gacagaggcg 8880

caggcccccc agaaccaggg ttgagggacg ccccgtcaaa gccagacaaa accaaggggt 8940

gttgagccca gcaagggaag gcccccaaac agaccaggag gtttctgaag gtgtctgtgt 9000

cacagtgggg catagccaca gctggtacca cagtgacact cacccagcca gaaaccccat 9060

tccaagtcag cggaagcaga gagagcaggg aggacacgtt taggatctga gactgcacct 9120

gacacccagg ccagcagacg tctcccctcc agggcacccc accctgtcct gcatttctgc 9180

aagatcaggg gcggcctgag ggggggtcta gggtgaggag atgggtcccc tgtacaccaa 9240

ggaggagtta ggcaggtccc gagcactc 9268

<210> SEQ ID NO 3
<211> LENGTH: 9441
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(9441)
<223> OTHER INFORMATION: HD 1.14-6.19

<400> SEQUENCE: 3

tccccattga ggctgacctg cccagagagt cctgggccca ccccacacac cggggcggaa 60

tgtgtgcagg cctcggtctc tgtgggtgtt ccgctagctg gggctcacag tgctcacccc 120

acacctaaaa tgagccacag cctccggagc ccccgcagga gaccccgccc acaagcccag 180

cccccaccca ggaggcccca gagctcaggg cgccccgtcg gattccgaac agccccgagt 240

cacagcgggt ataaccggaa ccaccactgt cagaatagct acgtcaaaaa ctgtccagtg 300

gccactgccg gaggccccgc cagagagggc agcagccact ctgatcccat gtcctgccgg 360

ctcccatgac ccccagcacg cggagcccca cagtgtcccc actggatggg aggacaagag 420

ctggggattc cggcgggtcg gggcaggggc ttgatcgcat ccttctgccg tggctccagt 480

gcccctggct ggagttgacc cttctgacaa gtgtcctcag agagacaggc atcaccggcg 540

cctcccaaca tcaaccccag gcagcacagg cacaaacccc acatccagag ccaactccag 600

-continued

gagcagagac accccaatac cctgggggac cccgaccctg atgacttccc actggaattc 660

gccgtagagt ccaccaggac caaagaccct gcctctgcct ctgtccctca ctcaggacct 720

gctgccgggc gaggccttgg gagcagactt gggcttaggg gacaccagtg tgaccccgac 780

cttgaccagg acgcagacct ttccttcctt tcctggggca gcacagactt tggggtctgg 840

gccaggagga acttctggca ggtcgccaag cacagaggcc acaggctgag gtggccctgg 900

aaagacctcc aggaggtggc cactcccctt cctcccagct ggaccccatg tcctccccaa 960

gataagggtg ccatccaagg caggtgctcc ttggagcccc attcagactc ctccctggac 1020

cccactgggc ctcagtccca gctctgggga tgaagccacc acaagcacac caggcagccc 1080

aggcccagcc accctgcagt gcccaagcac acactctgga gcagagcagg gtgcctctgg 1140

gaggggctga gctccccacc ccacccccac ctgcacaccc cacccacccc tgcccagcgg 1200

ctctgcagga gggtcagagc cccacatggg gtatggactt agggtctcac tcacgtggct 1260

cccatcatga gtgaaggggc ctcaagccca ggttcccaca gcagcgcctg tcgcaagtgg 1320

aggcagaggc ccgagggcca ccctgacctg gtccctgagg ttcctgcagc ccaggctgcc 1380

ctgctgtccc tgggaggcct gggctccacc agaccacagg tccagggcac cgggtgcagg 1440

agccacccac acacagctca caggaagaag ataagctcca gacccccagg gccagaacct 1500

gccttcctgc tactgcttcc tgccccagac ctgggcgccc tcccccgtcc acttacacac 1560

aggccaggaa gctgttccca cacagaacaa ccccaaacca ggaccgcctg gcactcaggt 1620

ggctgccatt tccttctcca tttgctccca gcgcctctgt cctccctggt tcctccttcg 1680

ggggaacagc ctgtgcagcc agtccctgca gcccacaccc tggggagacc caaccctgcc 1740

tggggccctt ccaaccctgc tgctcttact gcccacccag aaaactctgg ggtcctgtcc 1800

ctgcagtccc taccctggtc tccacccaga cccctgtgta tcactccaga cacccctccc 1860

aggcaaaccc tgcacctgca ggccctgtcc tcttctgtcg ctagagcctc agtttctccc 1920

ccctgtgccc acaccctacc tcctcctgcc cacaactcta actcttcttc tcctggagcc 1980

cctgagccat ggcattgacc ctgccctccc accacccaca gcccatgccc tcaccttcct 2040

cctggccact ccgaccccgc cccctctcag gccaagccct ggtatttcca ggacaaaggc 2100

tcacccaagt ctttcccagg caggcctggg ctcttgccct cacttcccgg ttacacggga 2160

gcctcctgtg cacagaagca gggagctcag cccttccaca ggcagaaggc actgaaagaa 2220

atcggcctcc agcaccttga cacacgtccg cccgtgtctc tcactgcccg cacctgcagg 2280

gaggctccgc actccctcta aagacaaggg atccaggcag cagcatcacg ggagaatgca 2340

gggctcccag acatcccagt cctctcacag gcctctcctg ggaagagacc tgcagccacc 2400

accaaacagc cacagaggct gctggatagt aactgagtca atgaccgacc tggagggcag 2460

gggagcagtg agccggagcc cataccatag ggacagagac cagccgctga catcccgagc 2520

tcctcaatgg tggccccata acacacctag gaaacataac acacccacag ccccacctgg 2580

aacagggcag agactgctga gcccccagca ccagccccaa gaaacaccag gcaacagtat 2640

cagaggggggc tcccgagaaa gagaggaggg gagatctcct tcaccatcaa atgcttccct 2700

tgaccaaaaa cagggtccac gcaactcccc caggacaaag gaggagcccc ctatacagca 2760

ctgggctcag agtcctctct gagacaccct gagtttcaga caacaacccg ctggaatgca 2820

cagtctcagc aggagaacag accaaagcca gcaaaaggga cctcggtgac accagtaggg 2880

acaggaggat tttgtggggg ctcgtgtcac tgtgaggaca ttgtagtcat ggtagctgcc 2940

-continued

actcccacag tgacacagac ccattcccaa agccctactg caaacacacc cactcctggg 3000

gctgaggggc tgggggagcg tctgggaagt agggtccagg ggtgtctatc aatgtccaaa 3060

atgcaccaga ctccccgcca aacaccaccc caccagccag cgagcagggt aaacagaaaa 3120

tgagaggctc tgggaagctt gcacaggccc caaggaaaga gctttggcgg gtgtgcaaga 3180

ggggatgcag gcagagcctg agcagggcct tttgctgttt ctgctttcct gtgcagagag 3240

ttccataaac tggtgttcaa gatcagtggc tgggaatgag cccaggaggg cagtctgtgg 3300

gaagagcaca gggaaggagg agcagccgct atcctacact gtcatctttc aaaagtttgc 3360

cttgtgacca cactattgca tcatgggatg cttaagagct gatgtagaca cagctaaaga 3420

gagaatcagt gagatgaatt tgcagcatag atctgaataa actctccaga atgtggagca 3480

gtacagaagc aaacacacag aaagtgcctg atgcaaggac aaagttcagt gggcaccttc 3540

aggcattgct gctgggcaca gacactctga aaagccttgg caggatctcc ctgcgacaaa 3600

gcagaaccct caggcaatgc cagccccaga gccctccctg agagcgtcat ggggaaagat 3660

gtgcagaaca gctgattatc atagactcaa actgagaaca gagcaaacgt ccatctgaag 3720

aacagtcaaa taagcaatgg taggttcatg caatgcaaac ccagacagcc aggggacaac 3780

agtagagggc tacaggcggc tttgcggttg agttcatgac aatgctgagt aattggagta 3840

acagaggaaa gcccaaaaaa tacttttaat gtgatttctt ctaaataaaa tttacaccag 3900

gcaaaatgaa ctgtcttctt aagggataaa ctttcccctg gaaaaactac aaggaaaatt 3960

aagaaaacga tgatcacata aacacagttg tggttacttc tactggggaa ggaagagggt 4020

atgagctgag acacacagag tcggcaagtc tccaagcaag cacagaacga atacattaca 4080

gtaccttgaa tacagcagtt aaacttctaa atcgcaagaa caggaaaatg cacacagctg 4140

tgtttagaaa attctcagtc cagcactatt cataatagca aagacattaa cccaggttgg 4200

ataaataaat gatgacacag gcaattgcac aatgatacag acatacattt agtacatgag 4260

acatcgatga tgtatcccca aagaaatgac tttaaagaga aaaggcctga tgtgtggtgg 4320

cactcacctc cctgggatcc ccggacaggt tgcaggcaca ctgtgtggca gggcaggctg 4380

gtacatgctg gcagctcctg gggcctgatg tggagcaagc gcagggctgt ataccccaa 4440

ggatggcaca gtcagtgaat tccagagaga agcagctcag ccacactgcc caggcagagc 4500

ccgagaggga cgcccacgta cagggaggca gagcccagct cctccacagc caccaccacc 4560

tgtgcacggg ccaccacctt gcaggcacag agtgggtgct gagaggaggg gcagggacac 4620

caggcagggt gagcacccag agaaaactgc agaagcctca cacatccacc tcagcctccc 4680

ctgacctgga cctcacctgg tctggacctc acctggcctg ggcctcacct gacctggacc 4740

tcacctggcc tgggcttcac ctgacctgga cctcacctgg cctccggcct cacctgcacc 4800

tgctccaggt cttgctggaa cctgagtagc actgaggctg cagaagctca tccagggttg 4860

gggaatgact ctggaactct cccacatctg acctttctgg gtggaggcat ctggtggccc 4920

tgggaatata aaaagcccca gaatggtgcc tgcgtgattt gggggcaatt tatgaacccg 4980

aaaggacatg gccatggggt gggtagggac atagggacag atgccagcct gaggtggagc 5040

ctcaggacac agttggacgc ggacactatc cacataagcg agggacagac ccgagtgttc 5100

ctgcagtaga cctgagagcg ctgggcccac agcctcccct cggtgccctg ctgcctcctc 5160

aggtcagccc tggacatccc gggtttcccc aggccagatg gtaggtttga agtgaggtct 5220

gtgtcactgt ggtatcatga tcacgtttgg gggagtcatc gttataccca cagcatcaca 5280

cggtccatca gaaacccatg ccacagccct ccccgcaggg gaccgccgcg tgccatgtta 5340

-continued

cgattttgat cgaggacaca gcgccatggg tatggtggct accacagcag tgcagcccat 5400

gacccaaaca cacagggcag caggcacaat ggacaggcct gtgagtgacc atgctgggct 5460

ccagcccgcc agccccggag accatgaaac agatggccaa ggtcacccca cagttcagcc 5520

agacatggct ccgtggggtc tgcatcgctg ctgccctcta acaccagccc agatggggac 5580

aaggccaacc ccacattacc atctcctgct gtccacccag tggtcccaga agcccctccc 5640

tcatggctga gccacatgtg tgaaccctga gagcacccca tgtcagagta ggggcagcag 5700

aagggcgggg ctggccctgt gcactgtccc tgcacccatg gtccctcgcc tgcctggccc 5760

tgacacctga gcctcttctg agtcatttct aagatagaag acattcccgg ggacagccgg 5820

agctgggcgt cgctcatccc gcccggccgt cctgagtcct gcttgtttcc agacctcacc 5880

agggaagcca acagaggact cacctcacac agtcagagac aaagaacctt ccagaaatcc 5940

ctgtctcact ccccagtggg caccttcttc caggacattc ctcggtcgca tcacagcagg 6000

cacccacatc tggatcagga cggcccccag aacacaagat ggcccatggg gacagcccca 6060

caacccaggc cttcccagac ccctaaaagg cgtcccaccc cctgcacctg ccccagggct 6120

aaaaatccag gaggcttgac tcccgcatac cctccagcca gacatcacct cagccccctc 6180

ctggagggga caggagcccg ggagggtgag tcagacccac ctgccctcga tggcaggcgg 6240

ggaagattca gaaaggcctg agatccccag gacgcagcac cactgtcaat gggggcccca 6300

gacgcctgga ccagggcctg cgtgggaaag gccgctgggc acactcaggg gctttttgtg 6360

aaggcccctc ctactgtgtg accacggtca ctaccacagt gatgaaacta gcagcaaaaa 6420

ctggccggac acccagggac catgcacact tctcagcttg gagctctcca ggaccagaag 6480

agtcaggtct gagggtttgt agccagaccc tcggcctcta gggacaccct ggccatcaca 6540

gcggatgggc tggtgcccca catgccatct gctccaaaca ggggcttcag agggctctga 6600

ggtgacttca ctcatgacca caggtgccct ggccccttcc ccgccagcta caccgaaccc 6660

tgtcccaaca gctgccccag ttccaacagc caattcctgg ggcccagaat tgctgtagac 6720

accagcctcg ttccagcacc tcctgccaat tgcctggatt cacatcctgg ctggaatcaa 6780

gagggcagca tccgccaggc tcccaacagg caggactccc gcacaccctc ctctgagagg 6840

ccgctgtgtt ccgcagggcc aggccctgga cagttcccct cacctgccac tagagaaaca 6900

cctgccattg tcgtccccac ctggaaaaga ccactcgtgg agccccccagc cccaggtaca 6960

gctgtagaga gactccccga gggatctaag aaggagccat gcgcagttct gccgggaccc 7020

tcggccaggc cgacaggagt ggacactgga gctgggccca cactgggcca cataggagct 7080

caccagtgag ggcaggagag cacatgccgg ggagcaccca gcctcctgct gaccagaggc 7140

ccgtcccaga gcccaggagg ctgcagaggc ctctccaggg ggacactgtg catgtctggt 7200

ccctgagcag ccccccacgt ccccagtcct gggggccccct ggcacagctg tctggaccct 7260

ccctgttccc tgggaagctc ctcctgacag ccccgcctcc agttccaggt gtggttattg 7320

tcaggggggtg tcagactgtg gtggacacag ccatggttac cacagtggtg ctgcccatag 7380

cagcaaccag gccaagtaga caggcccctg ctgtgcagcc ccaggcctcc acttcacctg 7440

cttctcctgg ggctctcaag gtcactgttg tctgtactct gccctctgtg gggagggttc 7500

cctcagtggg aggtctgttc tcaacatccc agggcctcat gtctgcacgg aaggccaatg 7560

gatgggcaac ctcacatgcc gcggctaaga tagggtgggc agcctggcgg gggacagtac 7620

atactgctgg ggtgtctgtc actgtgccta gtggggcact ggctcccaaa caacgcagtc 7680

-continued

ctcgccaaaa tccccacagc ctcccctgct aggggctggc ctgatctcct gcagtcctag 7740

gaggctgctg acctccagaa tgtctccgtc cccagttcca gggcgagagc agatcccagg 7800

ccggctgcag actgggaggc caccccctcc ttcccagggt tcactggagg tgaccaaggt 7860

aggaaatggc cttaacacag ggatgactgc gccatccccc aacagagtca gccccctcct 7920

gctctgtacc ccgcaccccc caggccagtc cacgaaaacc agggccccac atcagagtca 7980

ctgcctggcc cggccctggg gcggaccccc cagcccccac cctgtctaga ggacttgggg 8040

ggacaggaca caggccctct ccttatggtt cccccacctg cctccggccg ggacccttgg 8100

ggtgtggaca gaaaggacac ctgcctaatt ggcccccagg aacccagaac ttctctccag 8160

ggaccccagc ccgagcaccc ccttacccag gacccagccc tgcccctcct cccctctgct 8220

ctcctctcat caccccatgg gaatccggta tccccaggaa gccatcagga agggctgaag 8280

gaggaagcgg ggccgtgcac caccgggcag gaggctccgt cttcgtgaac ccagggaagt 8340

gccagcctcc tagagggtat ggtccaccct gcctggggct cccaccgtgg caggctgcgg 8400

ggaaggacca gggacggtgt gggggagggc tcagggccct gcgggtgctc ctccatcttc 8460

ggtgagcctc ccccttcacc caccgtcccg cccacctcct ctccaccctg gctgcacgtc 8520

ttccacacca tcctgagtcc tacctacacc agagccagca aagccagtgc agacaaaggc 8580

tggggtgcag gggggctgcc agggcagctt cggggaggga aggatggagg gaggggaggt 8640

cagtgaagag gccccccttcc cctgggtcca ggatcctcct ctgggacccc cggatcccat 8700

cccctcctgg ctctgggagg agaagcagga tgggagaatc tgtgcgggac cctctcacag 8760

tggaatatcc ccacagcggc tcaggccaga cccaaaagcc cctcagtgag ccctccactg 8820

cagtcctggg cctgggtagc agcccctccc acagaggaca gacccagcac cccgaagaag 8880

tcctgccagg gggagctcag agccatgaaa gagcaggata tggggtcccc gatacaggca 8940

cagacctcag ctccatccag gcccaccggg acccaccatg ggaggaacac ctgtctccgg 9000

gttgtgaggt agctggcctc tgtctcggac cccactccag acaccagaca gaggggcagg 9060

ccccccaaaa ccagggttga gggatgatcc gtcaaggcag acaagaccaa ggggcactga 9120

ccccagcaag ggaaggctcc caaacagacg aggaggtttc tgaagctgtc tgtatcacag 9180

tggggcatag ccatggctgg taccacagtg acactcgcca ggccagaaac cccgtcccaa 9240

gtcagcggaa gcagagagag cagggaggac acgtttagga tctgaggccg cacctgacac 9300

ccagggcagc agacgtctcc cctccagggc accctccacc gtcctgcgtt tcttcaagaa 9360

taggggcggc ctgagggggt ccagggccag gcgataggtc ccctctaccc caaggaggag 9420

ccaggcagga cccgagcacc g 9441

<210> SEQ ID NO 4
<211> LENGTH: 11592
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(11592)
<223> OTHER INFORMATION: HD 1.20-6.25, 1.26

<400> SEQUENCE: 4

tccccattga ggctgacctg cccagacggg cctgggccca ccccacacac cggggcggaa 60

tgtgtgcagg ccccagtctc tgtgggtgtt ccgctagctg gggcccccag tgctcacccc 120

acacctaaag cgagccccag cctccagagc cccctaagca ttccccgccc agcagcccag 180

cccctgcccc cacccaggag gccccagagc tcagggcgcc tggtcggatt ctgaacagcc 240

-continued

ccgagtcaca gtgggtatca ctggcacgac caccgtgaga aaaactgtgt ccaaaactga 300

ctcctggcag cagtcggagg ccccgccaga gaggggagca gccggcctga acccatgtcc 360

tgccggttcc catgaccccc agcacccaga gccccacggt gtccccgttg gataatgagg 420

acaagggctg ggggctccgg tggtttgcgg cagggacttg atcacatcct tctgctgtgg 480

ccccattgcc tctggctgga gttgaccctt ctgacaagtg tcctcagaaa gacagggatc 540

accggcacct cccaatatca accccaggca gcacagacac aaaccccaca tccagagcca 600

actccaggag cagagacacc ccaacactct gggggacccc aaccgtgata actccccact 660

ggaatccgcc ccagagtcta ccaggaccaa aggccctgcc ctgtctctgt ccctcactca 720

gggcctcctg cagggcgagc gcttgggagc agactcggtc ttaggggaca ccactgtggg 780

ccccaacttt gatgaggcca ctgacccttc cttcctttcc tggggcagca cagactttgg 840

ggtctgggca gggaagaact actggctggt ggccaatcac agagccccca ggccgaggtg 900

gccccaagaa ggccctcagg aggtggccac tccacttcct cccagctgga ccccaggtcc 960

tccccaagat aggggtgcca tccaaggcag gtcctccatg gagccccctt cagactcctc 1020

ccgggacccc actggacctc agtccctgct ctgggaatgc agccaccaca agcacaccag 1080

gaagcccagg cccagccacc ctgcagtggg caagcccaca ctctggagca gagcagggtg 1140

cgtctgggag gggctaacct ccccaccccc caccccccat ctgcacacag ccacctacca 1200

ctgcccagac cctctgcagg agggccaagc caccatgggg tatggactta gggtctcact 1260

cacgtgcctc ccctcctggg agaaggggcc tcatgcccag atccctgcag cactagacac 1320

agctggaggc agtggcccca gggccaccct gacctggcat ctaaggctgc tccagcccag 1380

acagcactgc cgttcctggg aagcctgggc tccaccagac cacaggtcca gggcacagcc 1440

cacaggagcc acccacacac agctcacagg aagaagataa gctccagacc ccagggcggg 1500

acctgccttc ctgccaccac ttacacacag gccagggagc tgttcccaca cagatcaacc 1560

ccaaaccggg actgcctggc actagggtca ctgccatttc cctctccatt ccctcccagt 1620

gcctctgtgc tccctccttc tggggaacac cctgtgcagc ccctccctgc agcccacacg 1680

ctggggagac cccaccctgc ctcgggcctt ttctacctgc tgcacttgcc gcccacccaa 1740

acaaccctgg gtacgtgacc ctgcagtcct caccctgatc tgcaaccaga cccctgtccc 1800

tccctctaaa caccctccc aggccaactc tgcacctgca ggccctccgc tcttctgcca 1860

caagagcctc aggttttcct acctgtgccc acccctaac ccctcctgcc cacaacttga 1920

gttcttcctc tcctggagcc cttgagccat ggcactgacc ctacactccc acccacacac 1980

tgcccatgcc atcaccttcc tcctggacac tctgaccccg ctcccctccc tctcagaccc 2040

ggccctggta tttccaggac aaaggctcac ccaagtcttc cccatgcagg cccttgccct 2100

cactgcctgg ttacacggga gcctcctgtg cgcagaagca gggagctcag ctcttccaca 2160

ggcagaaggc actgaaagaa atcagcctcc agtgccttga cacacgtccg cctgtgtctc 2220

tcactgcctg cacctgcagg gaggctccgc actccctcta aagatgaggg atccaggcag 2280

caacatcacg ggagaatgca gggctcccag acagcccagc cctctcgcag gcctctcctg 2340

ggaagagacc tgcagccacc actgaacagc cacggaggtc gctggatagt aaccgagtca 2400

gtgaccgacc tggagggcag gggagcagtg aaccggagcc cataccatag ggacagagac 2460

cagccgctaa catcccgagc ccctcactgg cggccccaga acaccccgtg gaaagagaac 2520

agacccacag tcccacctgg aacagggcag acactgctga gcccccagca ccagccccaa 2580

-continued

gaaacactag gcaacagcat cagaggggc tcctgagaaa gagaggaggg gaggtctcct 2640

tcaccatcaa atgcttccct tgaccaaaaa cagggtccac gcaactcccc caggacaaag 2700

gaggagcccc ctgtacagca ctgggctcag agtcctctct gagacaggct cagtttcaga 2760

caacaacccg ctggaatgca cagtctcagc aggagagcca ggccagagcc agcaagagga 2820

gactcggtga caccagtctc ctgtagggac aggaggattt tgtgggggtt cgtgtcactg 2880

tgagcacatt gtggtggtca ctgccattcc cacagtgaca caaccccatt cctaaagccc 2940

tactgcaaac gcacccactc ctgggactga ggggctgggg gagcgtctgg gaagtatggc 3000

ctaggggtgt ccatcaatgc ccaaaatgca ccagactctc cccaagacat caccccacca 3060

gccagtgagc agagtaaaca gaaaatgaga agcagctggg aagcttgcac aggccccaag 3120

gaaagagctt tggcaggtgt gcaagagggg atgtgggcag agcctcagca gggccttttg 3180

ctgtttctgc tttcctgtgc agagagttcc ataaactggt attcaagatc aatggctggg 3240

agtgagccca ggaggacagt gtgggaagag cacagggaag gaggagcagc cgctatccta 3300

cactgtcatc ttttgaaagt ttgccctgtg cccacaatgc tgcatcatgg gatgcttaac 3360

agctgatgta gacacagcta aagagagaat cagtgaaatg gatttgcagc acagatctga 3420

ataaatcctc cagaatgtgg agcagcacag aagcaagcac acagaaagtg cctgatgcca 3480

aggcaaagtt cagtgggcac cttcaggcat tgctgctggg cacagacact ctgaaaagca 3540

ctggcaggaa ctgcctgtga caaagcagaa ccctcaggca atgccagccc tagagccctt 3600

cctgagaacc tcatgggcaa agatgtgcag aacagctgtt tgtcatagcc ccaaactatg 3660

gggctggaca aagcaaacgt ccatctgaag gagaacagac aaataaacga tggcaggttc 3720

atgaaatgca aactaggaca gccagaggac aacagtagag agctacaggc ggctttgcgg 3780

ttgagttcat gacaatgctg agtaattgga gtaacagagg aaagcccaaa aaatactttt 3840

aatgtgattt cttctaaata aaatttacac ccggcaaaat gaactatctt cttaagggat 3900

aaactttccc ctggaaaaac tataaggaaa atcaagaaaa cgatgatcac ataaacacag 3960

tggtggttac ttctactggg gaaggaagag ggtatgagct gagacacaca gagtcggcaa 4020

gtctcctaac aagaacagaa caaatacatt acagtacctt gaaaacagca gttaaacttc 4080

taaatcgcaa gaagaggaaa atgcacacac ctgtgtttag aaaattctca gtccagcact 4140

gttcataata gcaaagacat taacccaggt tggataaata agcgatgaca caggcaattg 4200

cacaatgata cagacataca ttcagtatat gagacatcga tgatgtatcc ccaaagaaat 4260

gactttaaag agaaaaggcc tgatgtgtgg tggcaatcac ctccctgggc atccccggac 4320

aggctgcagg ctcactgtgt ggcagggcag gcaggcacct gctggcagct cctggggcct 4380

gatgtggagc aggcacagag ctgtatatcc ccaaggaagg tacagtcagt gcattccaga 4440

gagaagcaac tcagccacac tccctggcca gaacccaaga tgcacaccca tgcacaggga 4500

ggcagagccc agcacctccg cagccaccac cacctgcgca cgggccacca ccttgcaggc 4560

acagagtggg tgctgagagg aggggcaggg acaccaggca gggtgagcac ccagagaaaa 4620

ctgcagaagc ctcacacatc cctcacctgg cctgggcttc acctgacctg gacctcacct 4680

ggcctcgggc ctcacctgca cctgctccag gtcttgctgg agcctgagta gcactgaggc 4740

tgtagggact catccagggt tggggaatga ctctgcaact ctcccacatc tgacctttct 4800

gggtggaggc acctggtggc ccagggaata taaaaagccc cagaatgatg cctgtgtgat 4860

ttgggggcaa tttatgaacc cgaaaggaca tggccatggg gtgggtaggg acagtaggga 4920

cagatgtcag cctgaggtga agcctcagga cacaggtggg catggacagt gtccacctaa 4980

-continued

gcgagggaca gacccgagtg tccctgcagt agacctgaga gcgctgggcc cacagcctcc 5040

cctcggggcc ctgctgcctc ctcaggtcag ccctggacat cccgggtttc cccaggcctg 5100

gcggtaggtt tgaagtgagg tctgtgtcac tgtggtatca ctatcatagt agtggtcatt 5160

actaccacag tgtcacagag tccatcaaaa actcatgcct gggagcctcc caccacagcc 5220

ctccctgcgg gggaccgctg catgccgtgt taggattttg atcgaggaca cggcgccatg 5280

ggtatggtgg ctaccacagc agtgcagccc atgacccaaa cacacggggc agcagaaaca 5340

atggacaggc ccacaagtga ccatgatggg ctccagccca ccagccccag agaccatgaa 5400

acagatggcc aaggtcaccc tacaggtcat ccagatctgg ctccaagggg tctgcatcgc 5460

tgctgccctc ccaacgccaa accagatgga gacagggccg gccccatagc accatctgct 5520

gccgtccacc cagcagtccc ggaagcccct ccctgaacgc tgggccacgt gtgtgaaccc 5580

tgcgagcccc ccatgtcaga gtaggggcag caggagggcg gggctggccc tgtgcactgt 5640

cactgcccct gtggtccctg gcctgcctgg ccctgacacc tgagcctctc ctgggtcatt 5700

tccaagacat tcccagggac agccggagct gggagtcgct catcctgcct ggctgtcctg 5760

agtcctgctc atttccagac ctcaccaggg aagccaacag aggactcacc tcacacagtc 5820

agagacaacg aaccttccag aaatccctgt ttctctcccc agtgagagaa accctcttcc 5880

agggtttctc ttctctccca ccctcttcca ggacagtcct cagcagcatc acagcgggaa 5940

cgcacatctg gatcaggacg gcccccagaa cacgcgatgg cccatgggga cagcccagcc 6000

cttcccagac ccctaaaagg tatccccacc ttgcacctgc cccagggctc aaactccagg 6060

aggcctgact cctgcacacc ctcctgccag atatcacctc agccccctcc tggaggggac 6120

aggagcccgg gagggtgagt cagacccacc tgccctcaat ggcaggcggg gaagattcag 6180

aaaggcctga gatccccagg acgcagcacc actgtcaatg ggggccccag acgcctggac 6240

cagggcctgt gtgggaaagg cctctggcca cactcagggg ctttttgtga agggccctcc 6300

tgctgtgtga ccacggtggt cactcccaca gtgatgaaac cagcagcaaa aactgaccgg 6360

actcgcaggg tttatgcaca cttctcggct cggagctctc caggagcaca agagccaggc 6420

ccgagggttt gtgcccagac cctcggcctc tagggacacc cgggccatct tagccgatgg 6480

gctgatgccc tgcacaccgt gtgctgccaa acaggggctt cagagggctc tgaggtgact 6540

tcactcatga ccacaggtgc cctggtccct tcactgccag ctgcaccaga ccctgttccg 6600

agagatgccc cagttccaaa agccaattcc tggggccggg aattactgta gacaccagcc 6660

tcattccagt acctcctgcc aattgcctgg attcccatcc tggctggaat caagagggca 6720

gcatccgcca ggctcccaac aggcaggact cccacacacc ctcctctgag aggccgctgt 6780

gttccgcagg gccaggccgc agacagttcc cctcacctgc ccatgtagaa acacctgcca 6840

ttgtcgtccc cacctggcaa agaccacttg tggagccccc agccccaggt acagctgtag 6900

agagagtcct cgaggcccct aagaaggagc catgcccagt tctgccggga ccctcggcca 6960

ggccgacagg agtggacgct ggagctgggc ccacactggg ccacatagga gctcaccagt 7020

gagggcagga gagcacatgc cggggagcac ccagcctcct gctgaccaga gacccgtccc 7080

agagcccagg aggctgcaga ggcctctcca gggggacaca gtgcatgtct ggtccctgag 7140

cagcccccag gctctctagc actgggggcc cctggcacag ctgtctggac cctccctgtt 7200

ccctgggaag ctcctcctga cagccccgcc tccagttcca ggtgtggtta ttgtcagggg 7260

gtgccaggcc gtggtagaca tggccaccat taccacagtg gtgccgccca tagcagcaac 7320

-continued

caggccaagt agacagaccc ctgccacgca gccccaggcc tccagctcac ctgcttctcc 7380

tggggctctc aaggctgctg tctgccctct ggccctctgt ggggagggtt ccctcagtgg 7440

gaggtctgtg ctccagggca gggatgactg agatagaaat caaaggctgg cagggaaagg 7500

cagcttcccg ccctgagagg tgcaggcagc accacagagc catggagtca cagagccacg 7560

gagcccccag tgtgggcgtg tgagggtgct gggctcccgg caggcccagc cctgatgggg 7620

aagcctgccc cgtcccacag cccaggtccc caggggcagc aggcacagaa gctgccaagc 7680

tgtgctctac gatcctcatc cctccagcag catccactcc acagtgggga aactgagcct 7740

tggagaacca cccagccccc tggaaacaag gcggggagcc cagacagtgg gcccagagca 7800

ctgtgtgtat cctggcacta ggtgcaggga ccacccggag atccccatca ctgagtggcc 7860

agcctgcaga aggacccaac cccaaccagg ccgcttgatt aagctccatc cccctgtcct 7920

gggaacctct tcccagcgcc accaacagct cggcttccca ggccctcatc cctccaagga 7980

aggccaaagg ctgggcctgc caggggcaca gtaccctccc ttgccctggc taagacaggg 8040

tgggcagacg gctgcagata ggacatattg ctggggcatc ttgctctgtg actactgggt 8100

actggctctc aacgcagacc ctaccaaaat ccccactgcc tcccctgcta ggggctggcc 8160

tggtctcctc ctgctgtcct aggaggctgc tgacctccag gatggcttct gtccccagtt 8220

ctagggccag agcagatccc aggcaggctg taggctggga ggccaccct gtccttgccg 8280

aggttcagtg caggcaccca ggacaggaaa tggcctgaac acagggatga ctgtgccatg 8340

ccctacctaa gtccgccct ttctactctg caaccccac tccccaggtc agcccatgac 8400

gaccaacaac ccaacaccag agtcactgcc tggccctgcc ctggggagga cccctcagcc 8460

cccaccctgt ctagaggagt tgggggaca ggacacaggc tctctcctta tggttccccc 8520

acctggctcc tgccgggacc cttggggtgt ggacagaaag gacgcctgcc taattggccc 8580

ccaggaaccc agaacttctc tccagggacc ccagcccgag caccccctta cccaggaccc 8640

agccctgccc ctcctccct ctgctctcct ctcatcactc catgggaatc cagaatcccc 8700

aggaagccat caggaagggc tgaaggagga agcggggccg ctgcaccacc gggcaggagg 8760

ctccgtcttc gtgaacccag ggaagtgcca gcctcctaga gggtatggtc caccctgcct 8820

ggggctccca ccgtggcagg ctgcggggaa ggaccaggga cggtgtgggg gagggctcag 8880

ggccctgcag gtgctccatc ttggatgagc ccatccctct cacccaccga cccgcccacc 8940

tcctctccac cctggccaca cgtcgtccac accatcctga gtcccaccta caccagagcc 9000

agcagagcca gtgcagacag aggctggggt gcaggggggc cgccagggca gctttgggga 9060

gggaggaatg gaggaagggg aggtcagtga agaggccccc ctcccctggg tctaggatcc 9120

acctttggga cccccggatc ccatcccctc caggctctgg gaggagaagc aggatgggag 9180

attctgtgca ggaccctctc acagtggaat acctccacag cggctcaggc cagatacaaa 9240

agcccctcag tgagccctcc actgcagtgc agggcctggg ggcagcccct cccacagagg 9300

acagacccag caccccgaag aagtcctgcc agggggagct cagagccatg aaggagcaag 9360

atatggggac cccaatactg gcacagacct cagctccatc caggcccacc aggacccacc 9420

atgggtggaa cacctgtctc cggcccctgc tggctgtgag gcagctggcc tctgtctcgg 9480

acccccattc cagacaccag acagagggac aggccccca gaaccagtgt tgagggacac 9540

ccctgtccag ggcagccaag tccaagaggc gcgctgagcc cagcaaggga aggcccccaa 9600

acaaaccagg aggtttctga agctgtctgt gtcacagtcg ggcatagcca cggctaccac 9660

aatgacactg ggcaggacag aaaccccatc ccaagtcagc cgaaggcaga gagagcaggc 9720

-continued

aggacacatt taggatctga ggccacacct gacactcaag ccaacagatg tctcccctcc 9780

agggcgccct gccctgttca gtgttcctga gaaaacaggg gcagcctgag gggatccagg 9840

gccaggagat gggtcccctc taccccgagg aggagccagg cgggaatccc agccccctcc 9900

ccattgaggc catcctgccc agaggggccc ggacccaccc cacacaccca ggcagaatgt 9960

gtgcaggcct caggctctgt gggtgccgct agctggggct gccagtcctc accccacacc 10020

taaggtgagc cacagccgcc agagcctcca caggagaccc cacccagcag cccagcccct 10080

acccaggagg ccccagagct cagggcgcct gggtggattc tgaacagccc cgagtcacgg 10140

tgggtatcat gggagccact accactgtga gaaaagctat gtccaaaact gtctcccggc 10200

cactgctgga ggcccagcca gagaagggac cagccgcccg aacatacgac cttcccagac 10260

ctcatgaccc ccagcacttg gagctccaca gtgtccccat tggatggtga ggatgggggc 10320

cggggccatc tgcacctccc aacatcaccc ccaggcagca caggcacaaa ccccaaatcc 10380

agagccgaca ccaggaacac agacacccca ataccctggg ggaccctggc cctggtgact 10440

tcccactggg atccaccccc gtgtccacct ggatcaaaga ccccaccgct gtctctgtcc 10500

ctcactcagg gcctgctgag gggcgggtgc tttggagcag actcaggttt aggggccacc 10560

attgtggggc ccaacctcga ccaggacaca gatttttctt tcctgccctg gggcaacaca 10620

gactttgggg tctgtgcagg gaggaccttc tggaaagtca ccaagcacag agccctgact 10680

gaggtggtct caggaagacc cccaggaggg ggcttgtgcc ccttcctctc atgtggaccc 10740

catgcccccc aagatagggg catcatgcag ggcaggtcct ccatgcagcc accactaggc 10800

aactccctgg cgccggtccc cactgcgcct ccatcccggc tctggggatg cagccaccat 10860

ggccacacca ggcagcccgg gtccagcaac cctgcagtgc ccaagccctt ggcaggattc 10920

ccagaggctg gagcccaccc ctcctcatcc ccccacacct gcacacacac acctaccccc 10980

tgcccagtcc ccctccagga gggttggagc cgcccatagg gtgggggctc caggtctcac 11040

tcactcgctt cccttcctgg gcaaaggagc ctcgtgcccc ggtcccccct gacggcgctg 11100

ggcacaggtg tgggtactgg gccccagggc tcctccagcc ccagctgccc tgctctccct 11160

gggaggcctg ggcaccacca gaccaccagt ccagggcaca gccccaggga gccgcccact 11220

gccagctcac aggaagaaga taagcttcag accctcaggg ccgggagctg ccttcctgcc 11280

accccttcct gccccagacc tccatgccct cccccaacca cttacacaca agccagggag 11340

ctgtttccac acagttcaac cccaaaccag gacggcctgg cactcgggtc actgccattt 11400

ctgtctgcat tcgctcccag cgcccctgtg ttccctccct cctccctcct tcctttcttc 11460

ctgcattggg ttcatgccgc agagtgccag gtgcaggtca gccctgagct tggggtcacc 11520

tcctcactga aggcagcctc agggtgccca ggggcaggca gggtgggggt gaggcttcca 11580

gctccaaccg ct 11592

<210> SEQ ID NO 5
<211> LENGTH: 689
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(689)
<223> OTHER INFORMATION: Mouse Ei (an intronic enhancer)

<400> SEQUENCE: 5

tctagagagg tctggtggag cctgcaaaag tccagctttc aaaggaacac agaagtatgt 60

-continued

gtatggaata ttagaagatg ttgcttttac tcttaagttg gttcctagga aaaatagtta 120

aatactgtga ctttaaaatg tgagagggtt ttcaagtact catttttta aatgtccaaa 180

attcttgtca atcagtttga ggtcttgttt gtgtagaact gatattactt aaagtttaac 240

cgaggaatgg gagtgaggct ctctcataac ctattcagaa ctgactttta acaataataa 300

attaagtttc aaatattttt aaatgaattg agcaatgttg agttggagtc aagatggccg 360

atcagaacca gaacacctgc agcagctggc aggaagcagg tcatgtggca aggctatttg 420

gggaagggaa aataaaacca ctaggtaaac ttgtagctgt ggtttgaaga agtggttttg 480

aaacactctg tccagcccca ccaaaccgaa agtccaggct gagcaaaaca ccacctgggt 540

aatttgcatt tctaaaataa gttgaggatt cagccgaaac tggagaggtc ctcttttaac 600

ttattgagtt caacctttta attttagctt gagtagttct agtttcccca aacttaagtt 660

tatcgacttc taaaatgtat ttagaattc 689

<210> SEQ ID NO 6
<211> LENGTH: 3518
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(3518)
<223> OTHER INFORMATION: Mouse Switch Region

<400> SEQUENCE: 6

acttatttca gttgaacatg ctggttggtg gttgagagga cactcagtca gtcagtgacg 60

tgaagggctt ctaagccagt ccacatgctc tgtgtgaact ccctctggcc ctgcttattg 120

ttgaatgggc caaaggtctg agaccaggct gctgctgggt aggcctggac tttgggtctc 180

ccacccagac ctgggaatgt atggttgtgg cttctgccac ccatccacct ggctgctcat 240

ggaccagcca gcctcggtgg ctttgaagga acaattccac acaaagactc tggacctctc 300

cgaaaccagg caccgcaaat ggtaagccag aggcagccac agctgtggct gctgctctta 360

aagcttgtaa actgtttctg cttaagaggg actgagtctt cagtcattgc tttaggggga 420

gaaagagaca tttgtgtgtc ttttgagtac cgttgtctgg gtcactcaca tttaactttc 480

cttgaaaaac tagtaaaaga aaaatgttgc ctgttaacca ataatcatag agctcatggt 540

actttgagga aatcttagaa agcgtgtata caattgtctg gaattatttc agttaagtgt 600

attagttgag gtactgatgc tgtctctact tcagttatac atgtgggttt gaattttgaa 660

tctattctgg ctcttcttaa gcagaaaatt tagataaaat ggatacctca gtggttttta 720

atggtgggtt taatatagaa ggaatttaaa ttggaagcta atttagaatc agtaaggagg 780

gacccaggct aagaaggcaa tcctgggatt ctggaagaaa agatgttttt agtttttata 840

gaaaacacta ctacattctt gatctacaac tcaatgtggt ttaatgaatt tgaagttgcc 900

agtaaatgta cttcctggtt gttaaagaat ggtatcaaag gacagtgctt agatccgagg 960

tgagtgtgag aggacagggg ctggggtatg gatacgcaga aggaaggcca cagctgtaca 1020

gaattgagaa agaatagaga cctgcagttg aggccagcag gtcggctgga ctaactctcc 1080

agccacagta atgacccaga cagagaaagc cagactcata aagcttgctg agcaaaatta 1140

agggaacaag gttgagagcc ctagtaagcg aggctctaaa aagcacagct gagctgagat 1200

gggtgggctt ctctgagtgc ttctaaaatg cgctaaactg aggtgattac tctgaggtaa 1260

gcaaagctgg gcttgagcca aaatgaagta gactgtaatg aactggaatg agctgggccg 1320

ctaagctaaa ctaggctggc ttaaccgaga tgagccaaac tggaatgaac ttcattaatc 1380

-continued

taggttgaat agagctaaac tctactgcct acactggact gttctgagct gagatgagct 1440

ggggtgagct cagctatgct acgctgtgtt ggggtgagct gatctgaaat gagatactct 1500

ggagtagctg agatggggtg agatggggtg agctgagctg ggctgagcta gactgagctg 1560

agctagggtg agctgagctg ggtgagctga gctaagctgg ggtgagctga gctgagcttg 1620

gctgagctag ggtgagctgg gctgagctgg ggtgagctga gctgagctgg ggtaagctgg 1680

gatgagctgg ggtgagctga gctgagctgg agtgagctga gctgggctga gctggggtga 1740

gctgggctga gctgggctga gctgggctga gctggggtga gctgagctgg ggtgagctga 1800

gctgagctgg ggtgagctga gctgagctgg ggtgagctgg ggtgagctga gctggggtga 1860

gctgagctga gctggggtga gctgagctgg ggtgagctga gctgagctgg ggtgagctga 1920

gctgagctga gctgagctga gctggggtga gctgagctga gctgagctgg ggtgagctgg 1980

ggtgagctga gctgagctgg agtgagctga gctgggctga gctggggtga gctgggctga 2040

gctggggtga gctgagctga gctgagctga gctggggtga gctgagctga gctggggtga 2100

gctgagctgg ggtgagctgg gctgagctga gctgagctga gctgagctga gctgagctga 2160

gctgagctga gctgagctga gctgagctga gctgagctga gctgagctgg ggtgagctga 2220

gctgagctgg gctgagctgg ggtgagctgg gctgagctgg gctgagctgg gctgagctgg 2280

ggtgagctga gctggggtga gctgagctga gctgggctga gctgagctga gctggggtga 2340

gctgagctga gctggggtga gctgagctga gctgagctgg ggtgagctga gctgagctgg 2400

gctgagcagg gctgagctgg ggtgagctga gctgagctgg ggtgagctgg gctgagctgg 2460

gctgagctga gctgagctgg gctgagctgg gctgagctgg gctgagctgg gctgagctgg 2520

gctgagctgg ggtgagctga gctggggtga gctggggtga gctgagctgg ggtgagctga 2580

gctggggtga gctgagctga gctggggtga gctgagctgg ggtgagctga gctgagctgg 2640

ggtgagctga gctgagctgg ggtgagctga gctagggtga actgggctgg gtgagctgga 2700

gtgagctgag ctgaggtgaa ctggggtgag ccgggatgtt ttgagttgag ctggggtaag 2760

atgagctgaa ctggggtaaa ctgggatgag ctgtggtgag cggagctgga ttgaactgag 2820

ctgtgtgagc tgagctgggg tcagctgagc aagagtgagt agagctggct ggccagaacc 2880

agaatcaatt aggctaagtg agccagattg tgctgggatc agctgtactc agatgagctg 2940

ggatgaggta ggctgggatg agctgggcta gctgacatgg attatgtgag gctgagctag 3000

catgggctgg cctagctgat gagctaagct tgaatgagcg gggctgagct ggactcagat 3060

gtgctagact gagctgtact ggatgatctg gtgtagggtg atctggactc aactgggctg 3120

gctgatggga tgcgccaggt tgaactaggc tcagataagt taggctgagt agggcctggt 3180

tgagatggtt cgggatgagc tgggaaaaga tggactcgga ccatgaactg ggctgagctg 3240

ggttgggaga ccatgaattg agctgaactg agtgcagctg ggataaactg ggttgagcta 3300

agaatagact acctgaattg tgccaaactc ggctgggatc aattggaaat tatcaggatt 3360

tagatgagcc ggactaaact atgctgagct ggactggttg gatgtgttga actggcctgc 3420

tgctgggctg gcatagctga gttgaactta aatgaggaag gctgagcaag gctagcctgc 3480

ttgcatagag ctgaacttta gcctagcctg agctggac 3518

<210> SEQ ID NO 7
<211> LENGTH: 315
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:

-continued

<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(315)
<223> OTHER INFORMATION: mouse IgM exon 1

<400> SEQUENCE: 7

agagtcagtc cttcccaaat gtcttccccc tcgtctcctg cgagagcccc ctgtctgata 60

agaatctggt ggccatgggc tgcctggccc gggacttcct gcccagcacc atttccttca 120

cctggaacta ccagaacaac actgaagtca tccagggtat cagaaccttc ccaacactga 180

ggacaggggg caagtaccta gccacctcgc aggtgttgct gtctcccaag agcatccttg 240

aaggttcaga tgaatacctg gtatgcaaaa tccactacgg aggcaaaaac aaagatctgc 300

atgtgcccat tccag 315

<210> SEQ ID NO 8
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Val Gln Leu Glu Arg
1 5

<210> SEQ ID NO 9
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 9

Val Pro Leu Ala Arg
1 5

<210> SEQ ID NO 10
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 10

Val Ser Leu Ala Leu
1 5

<210> SEQ ID NO 11
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 11

Val Ser Leu Ala Arg
1 5

<210> SEQ ID NO 12
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Trp Glu Leu Leu
1

<210> SEQ ID NO 13
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 13

Val Ser Trp Glu Pro Leu
1 5

<210> SEQ ID NO 14
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Tyr Gln Leu Leu Tyr
1 5

<210> SEQ ID NO 15
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 15

Ser Tyr Gln Leu Pro Tyr
1 5

<210> SEQ ID NO 16
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Arg Ile Leu Tyr
1

<210> SEQ ID NO 17
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Trp Cys Met Leu Tyr
1 5

<210> SEQ ID NO 18
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 18

Arg Thr Leu Tyr Ser Trp Cys Met Pro Tyr
1 5 10

<210> SEQ ID NO 19
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

-continued

Ser Ile Leu Trp Trp
1 5

<210> SEQ ID NO 20
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 20

Ser Thr Leu Trp Trp Ser Leu Pro Phe
1 5

<210> SEQ ID NO 21
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Val Leu Arg Phe Leu Glu Trp Leu Leu Tyr
1 5 10

<210> SEQ ID NO 22
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 22

Val Ser Pro Phe Leu Glu Trp Ser Leu Tyr
1 5 10

<210> SEQ ID NO 23
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Val Leu Arg Tyr Phe Asp Trp Leu Leu
1 5

<210> SEQ ID NO 24
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 24

Val Ser Pro Tyr Phe Asp Trp Ser Leu
1 5

<210> SEQ ID NO 25
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

Val Leu Leu Trp Phe Gly Glu Leu Leu
1 5

<210> SEQ ID NO 26
<211> LENGTH: 9

-continued

<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 26

Val Ser Pro Trp Phe Gly Glu Ser Leu
1 5

<210> SEQ ID NO 27
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

Leu Arg Leu Gly Glu Leu Ser Leu Tyr
1 5

<210> SEQ ID NO 28
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 28

Ser Arg Leu Gly Glu Ser Ser Leu Tyr
1 5

<210> SEQ ID NO 29
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 29

Trp Leu Leu Leu
1

<210> SEQ ID NO 30
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 30

Val Ser Leu Ser
1

<210> SEQ ID NO 31
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 31

Trp Ser Leu Leu
1

<210> SEQ ID NO 32
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

-continued

<400> SEQUENCE: 32

Pro Gln Ser Leu
1

<210> SEQ ID NO 33
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 33

Pro Arg Ser Leu
1

<210> SEQ ID NO 34
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 34

Pro Arg Trp Ser Leu
1 5

<210> SEQ ID NO 35
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

Trp Ile Gln Leu Trp Leu
1 5

<210> SEQ ID NO 36
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 36

Trp Thr Gln Pro Trp Leu
1 5

<210> SEQ ID NO 37
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 37

Trp Leu Arg Leu
1

<210> SEQ ID NO 38
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 38

Trp Pro Pro Leu
1

<210> SEQ ID NO 39
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 39

Arg Trp Leu Gln Leu
1 5

<210> SEQ ID NO 40
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 40

Thr Trp Pro Pro Leu
1 5

<210> SEQ ID NO 41
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 41

Gln Gln Leu Val
1

<210> SEQ ID NO 42
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 42

Pro Gln Leu Val
1

<210> SEQ ID NO 43
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 43

Gln Trp Leu Val
1

<210> SEQ ID NO 44
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 44

Pro Trp Leu Val
1

<210> SEQ ID NO 45
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 45

Tyr Asn Trp Asn Asp
1 5

<210> SEQ ID NO 46
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 46

Tyr His Trp His Asp
1 5

<210> SEQ ID NO 47
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

Tyr Asn Trp Asn Tyr
1 5

<210> SEQ ID NO 48
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 48

Tyr His Trp His Tyr
1 5

<210> SEQ ID NO 49
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

Tyr Ser Gly Ser Tyr Tyr
1 5

<210> SEQ ID NO 50
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 50

Tyr His Gly Ser His Tyr
1 5

<210> SEQ ID NO 51
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 51

Gly Tyr Cys Ser Ser Thr Ser Cys Tyr Thr
1 5 10

<210> SEQ ID NO 52
<211> LENGTH: 10

-continued

<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 52

Gly His Cys Ser His Thr Ser Cys His Thr
1 5 10

<210> SEQ ID NO 53
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 53

Gly Tyr Cys Thr Asn Gly Val Cys Tyr Thr
1 5 10

<210> SEQ ID NO 54
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 54

Gly His Cys Thr His Gly Val Cys His Thr
1 5 10

<210> SEQ ID NO 55
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 55

Gly Tyr Cys Ser Gly Gly Ser Cys Tyr Ser
1 5 10

<210> SEQ ID NO 56
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 56

Gly His Cys Ser His Gly Ser Cys His Ser
1 5 10

<210> SEQ ID NO 57
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 57

Ala Tyr Cys Gly Gly Asp Cys Tyr Ser
1 5

<210> SEQ ID NO 58
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 58

-continued

Ala His Cys Gly Gly His Cys His Ser
1 5

<210> SEQ ID NO 59
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 59

Tyr Tyr Asp Phe Trp Ser Gly Tyr Tyr Thr
1 5 10

<210> SEQ ID NO 60
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 60

Tyr His His Phe Trp Ser Gly His Tyr Thr
1 5 10

<210> SEQ ID NO 61
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 61

Tyr Tyr Asp Ile Leu Thr Gly Tyr Tyr Asn
1 5 10

<210> SEQ ID NO 62
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 62

Tyr His His Ile Leu Thr Gly His Tyr Asn
1 5 10

<210> SEQ ID NO 63
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 63

Tyr Tyr Tyr Gly Ser Gly Ser Tyr Tyr Asn
1 5 10

<210> SEQ ID NO 64
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 64

Tyr His His Gly Ser Gly Ser His Tyr Asn
1 5 10

<210> SEQ ID NO 65
<211> LENGTH: 12
<212> TYPE: PRT

-continued

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 65

Tyr Tyr Asp Tyr Val Trp Gly Ser Tyr Arg Tyr Thr
1 5 10

<210> SEQ ID NO 66
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 66

Tyr His Asp His Val Trp Gly Ser His Arg Tyr Thr
1 5 10

<210> SEQ ID NO 67
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 67

Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
1 5 10

<210> SEQ ID NO 68
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 68

Tyr His Tyr His Ser Ser Gly His Tyr Tyr
1 5 10

<210> SEQ ID NO 69
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 69

Asp Tyr Ser Asn Tyr
1 5

<210> SEQ ID NO 70
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 70

Pro Gln Ser Leu
1

<210> SEQ ID NO 71
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 71

Asp Tyr Gly Asp Tyr
1 5

-continued

<210> SEQ ID NO 72
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 72

Asp His Gly His Tyr
1 5

<210> SEQ ID NO 73
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 73

Asp Tyr Gly Gly Asn Ser
1 5

<210> SEQ ID NO 74
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 74

Asp His Gly Gly His Ser
1 5

<210> SEQ ID NO 75
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 75

Gly Tyr Ser Tyr Gly Tyr
1 5

<210> SEQ ID NO 76
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 76

Gly His Ser His Gly Tyr
1 5

<210> SEQ ID NO 77
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 77

Gly Tyr Ser Gly Tyr Asp Tyr
1 5

<210> SEQ ID NO 78
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 78

Gly His Ser Gly His His Tyr
1 5

<210> SEQ ID NO 79
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 79

Arg Asp Gly Tyr Asn Tyr
1 5

<210> SEQ ID NO 80
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 80

Arg His Gly His His Tyr
1 5

<210> SEQ ID NO 81
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 81

Glu Tyr Ser Ser Ser Ser
1 5

<210> SEQ ID NO 82
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 82

Glu His Ser His Ser Ser
1 5

<210> SEQ ID NO 83
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 83

Gly Tyr Ser Ser Ser Trp Tyr
1 5

<210> SEQ ID NO 84
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 84

Gly His Ser His Ser Trp Tyr
1 5

-continued

<210> SEQ ID NO 85
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 85

Gly Tyr Ser Ser Gly Trp Tyr
1 5

<210> SEQ ID NO 86
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 86

Gly His Ser His Gly Trp Tyr
1 5

<210> SEQ ID NO 87
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 87

Gly Tyr Ser Ser Gly Tyr
1 5

<210> SEQ ID NO 88
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 88

Gly Thr Thr Gly Thr
1 5

<210> SEQ ID NO 89
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 89

Gly Ile Thr Gly Thr
1 5

<210> SEQ ID NO 90
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 90

Gly Ile Val Gly Ala Thr
1 5

<210> SEQ ID NO 91
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 91

Gly Ile Met Gly Ala Thr
1 5

<210> SEQ ID NO 92
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 92

Asp Ile Val Val Val Pro Ala Ala Ile
1 5

<210> SEQ ID NO 93
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 93

Asp Ile Val Val Ile Pro Ala Ala Ile
1 5

<210> SEQ ID NO 94
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 94

Asp Ile Val Leu Met Val Tyr Ala Ile
1 5

<210> SEQ ID NO 95
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 95

Asp Ile Val Val Val Val Ala Ala Thr
1 5

<210> SEQ ID NO 96
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 96

Asp Ile Val Val Met Val Ala Ala Thr
1 5

<210> SEQ ID NO 97
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 97

His Ile Val Val Val Thr Ala Ile
1 5

<210> SEQ ID NO 98
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 98

-continued

Ile Thr Ile Phe Gly Val Val Ile Ile
1 5

<210> SEQ ID NO 99
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 99

Ile Thr Ile Phe
1

<210> SEQ ID NO 100
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 100

Leu Val Ile Ile
1

<210> SEQ ID NO 101
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 101

Ile Thr Met Val Arg Gly Val Ile Ile
1 5

<210> SEQ ID NO 102
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 102

Ile Met Ile Thr Phe Gly Gly Val Ile Val Ile
1 5 10

<210> SEQ ID NO 103
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 103

Ile Thr Met Ile Val Val Val Ile Thr
1 5

<210> SEQ ID NO 104
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 104

Ile Thr Ile Val Val Val Ile Thr
1 5

<210> SEQ ID NO 105
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 105

Thr Thr Val Thr
1

<210> SEQ ID NO 106
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 106

Thr Thr Val Val Thr
1 5

<210> SEQ ID NO 107
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 107

Val Asp Thr Ala Met Val
1 5

<210> SEQ ID NO 108
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 108

Val Asp Ile Val Ala Thr Ile
1 5

<210> SEQ ID NO 109
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 109

Val Glu Met Ala Thr Ile
1 5

<210> SEQ ID NO 110
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 110

Val Asp Met Ala Thr Ile
1 5

<210> SEQ ID NO 111
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 111

Ser Ile Ala Ala Arg
1 5

<210> SEQ ID NO 112
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

-continued

<400> SEQUENCE: 112

Ser Ile Ala Thr Arg
1 5

<210> SEQ ID NO 113
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 113

Gly Ile Ala Ala Ala Gly
1 5

<210> SEQ ID NO 114
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 114

Gly Ile Ala Thr Ala Gly
1 5

<210> SEQ ID NO 115
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 115

Gly Ile Ala Val Ala Gly
1 5

<210> SEQ ID NO 116
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 116

Gly Ile Ala Met Ala Gly
1 5

<210> SEQ ID NO 117
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 117

Gly Ile Ala Ala Ala
1 5

<210> SEQ ID NO 118
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 118

Gly Ile Ala Thr Ala
1 5

<210> SEQ ID NO 119

<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 119

cgggtcactg ccatttctg 19

<210> SEQ ID NO 120
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 120

tctgcattcg ctcccagcgc 20

<210> SEQ ID NO 121
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 121

tctgcggcat gaacccaat 19

<210> SEQ ID NO 122
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 122

gtgcagggag gaccttctg 19

<210> SEQ ID NO 123
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 123

agtcaccaag cacagagccc tgac 24

<210> SEQ ID NO 124
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 124

gccagggagt tgcctagtg 19

<210> SEQ ID NO 125
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 125

-continued

gtggcccact tcccttcct 19

<210> SEQ ID NO 126
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 126

cagctggaac ccaccatgac ct 22

<210> SEQ ID NO 127
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 127

gacctgcctc ggatgaca 18

<210> SEQ ID NO 128
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 128

tggccagaac tgaccctac 19

<210> SEQ ID NO 129
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 129

accgacaaga gtccctcagg 20

<210> SEQ ID NO 130
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 130

ggagtcggct ctggatgtg 19

<210> SEQ ID NO 131
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 131

tgcggccgat cttagcc 17

<210> SEQ ID NO 132
<211> LENGTH: 21
<212> TYPE: DNA

-continued

<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 132

acgagcgggt tcggcccatt c 21

<210> SEQ ID NO 133
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 133

ttgaccgatt ccttgcgg 18

<210> SEQ ID NO 134
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 134

cagtcccgtt gatccagcc 19

<210> SEQ ID NO 135
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 135

cccatcaggg attttgtatc tctgtggacg 30

<210> SEQ ID NO 136
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 136

ggatatgcag cactgtgcca c 21

<210> SEQ ID NO 137
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 137

tcctccaacg acaggtccc 19

<210> SEQ ID NO 138
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 138

tccctggaac tctgccccga caca 24

<210> SEQ ID NO 139
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 139

gatgaactga cgggcacagg 20

<210> SEQ ID NO 140
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 140

atcacactca tcccatcccc 20

<210> SEQ ID NO 141
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 141

cccttcccta agtaccacag agtgggctc 29

<210> SEQ ID NO 142
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 142

cacagggaag caggaactgc 20

<210> SEQ ID NO 143
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 143

ggagccaggc aggacaca 18

<210> SEQ ID NO 144
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 144

tgggctcgta gtttgacgt 19

<210> SEQ ID NO 145
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 145

gggactttct tacccacact tca 23

<210> SEQ ID NO 146
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 146

ggtcccgagc actcttaatt aaac 24

<210> SEQ ID NO 147
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 147

cctcgaatgg aactac 16

<210> SEQ ID NO 148
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 148

gggagagcaa ccattcgttg t 21

<210> SEQ ID NO 149
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 149

ccgagcaccg atgcatcta 19

<210> SEQ ID NO 150
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 150

cgcagtcatg taatgc 16

<210> SEQ ID NO 151
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 151

gggaggcgaa ctgactgtca 20

-continued

<210> SEQ ID NO 152
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 152

ggtggagagg ctattcggc 19

<210> SEQ ID NO 153
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 153

tgggcacaac agacaatcgg ctg 23

<210> SEQ ID NO 154
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 154

gaacacggcg gcatcag 17

<210> SEQ ID NO 155
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 155

ggtacaactg gaacgac 17

<210> SEQ ID NO 156
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 156

ggtataactg gaactac 17

<210> SEQ ID NO 157
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 157

ggtataaccg gaaccac 17

<210> SEQ ID NO 158
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 158

Tyr Asn Arg Asn His
1 5

<210> SEQ ID NO 159
<211> LENGTH: 17

-continued

<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 159

ggtataactg gaacgac 17

<210> SEQ ID NO 160
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 160

ggtatagtgg gagctactac 20

<210> SEQ ID NO 161
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 161

aggatattgt agtagtacca gctgctatgc c 31

<210> SEQ ID NO 162
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 162

Tyr Gln Leu Leu Cys
1 5

<210> SEQ ID NO 163
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 163

Gly Tyr Cys Ser Ser Thr Ser Cys Tyr Ala
1 5 10

<210> SEQ ID NO 164
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 164

Asp Ile Val Val Val Pro Ala Ala Met
1 5

<210> SEQ ID NO 165
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 165

aggatattgt agtagtacca gctgctatac c 31

<210> SEQ ID NO 166
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 166

tggatattgt agtagtacca gctgctatgc c 31

<210> SEQ ID NO 167
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 167

Tyr Gln Leu Leu Cys
1 5

<210> SEQ ID NO 168
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 168

Gly Tyr Cys Ser Ser Thr Ser Cys Tyr Ala
1 5 10

<210> SEQ ID NO 169
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 169

Asp Ile Val Val Val Pro Ala Ala Met
1 5

<210> SEQ ID NO 170
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 170

aggatattgt actaatggtg tatgctatac c 31

<210> SEQ ID NO 171
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 171

aagatattgt actggtggtg tatgctatac c 31

<210> SEQ ID NO 172
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 172

Arg Ile Leu Tyr Trp Trp Cys Met Leu Tyr
1 5 10

<210> SEQ ID NO 173
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 173

Gly Tyr Cys Thr Gly Gly Val Cys Tyr Thr
1 5 10

<210> SEQ ID NO 174
<211> LENGTH: 9

-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 174

Asp Ile Val Leu Val Val Tyr Ala Ile
1 5

<210> SEQ ID NO 175
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 175

aggatattgt agtggtggta gctgctactc c 31

<210> SEQ ID NO 176
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 176

agcatattgt ggtggtgatt gctattcc 28

<210> SEQ ID NO 177
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 177

His Ile Val Val Val Ile Ala Ile
1 5

<210> SEQ ID NO 178
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 178

agcatattgt ggtggtgact gctattcc 28

<210> SEQ ID NO 179
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 179

gtattacgat ttttggagtg gttattatac c 31

<210> SEQ ID NO 180
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 180

gtattagcat ttttggagtg gttattatac c 31

<210> SEQ ID NO 181
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 181

-continued

Val Leu Ala Phe Leu Glu Trp Leu Leu Tyr
1 5 10

<210> SEQ ID NO 182
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 182

His Phe Trp Ser Gly Tyr Tyr Thr
1 5

<210> SEQ ID NO 183
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 183

Ile Ser Ile Phe Gly Val Val Ile Ile
1 5

<210> SEQ ID NO 184
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 184

gtattacgat attttgactg gttattataa c 31

<210> SEQ ID NO 185
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 185

gtattactat ggttcgggga gttattataa c 31

<210> SEQ ID NO 186
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 186

gtattactat gttcggggag ttattataac 30

<210> SEQ ID NO 187
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 187

Val Leu Leu Cys Ser Gly Ser Tyr Tyr Asn
1 5 10

<210> SEQ ID NO 188
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 188

Tyr Tyr Tyr Val Arg Gly Val Ile Ile
1 5

<210> SEQ ID NO 189

<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 189

Ile Thr Met Phe Gly Arg Leu Leu
1 5

<210> SEQ ID NO 190
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 190

gtattatgat tacgtttggg ggagttatgc ttatacc 37

<210> SEQ ID NO 191
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 191

Leu Arg Leu Gly Glu Leu Cys Leu Tyr
1 5

<210> SEQ ID NO 192
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 192

Tyr Tyr Asp Tyr Val Trp Gly Ser Tyr Ala Tyr Thr
1 5 10

<210> SEQ ID NO 193
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 193

Ile Met Ile Thr Phe Gly Gly Val Met Leu Ile
1 5 10

<210> SEQ ID NO 194
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 194

gtattactat gatagtagtg gttattacta c 31

<210> SEQ ID NO 195
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 195

tgactacagt aactac 16

<210> SEQ ID NO 196
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 196

tgactacggt gactac 16

<210> SEQ ID NO 197
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 197

tgactacggt ggtaactcc 19

<210> SEQ ID NO 198
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 198

gtggatacag ctatggttac 20

<210> SEQ ID NO 199
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 199

gtggatatag tggctacgat tac 23

<210> SEQ ID NO 200
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 200

gtagagatgg ctacaattac 20

<210> SEQ ID NO 201
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 201

gagtatagca gctcgtcc 18

<210> SEQ ID NO 202
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 202

gggtatagca gcagctggta c 21

<210> SEQ ID NO 203
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 203

gggtatagca gtggctggta c 21

<210> SEQ ID NO 204
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 204

gggtatagca gcggctac 18

<210> SEQ ID NO 205
<211> LENGTH: 11
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 205

ctaactgggg a 11

<210> SEQ ID NO 206
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 206

gtcgttccag ttgtacc 17

<210> SEQ ID NO 207
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 207

Val Val Pro Val Val
1 5

<210> SEQ ID NO 208
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 208

Ser Phe Gln Leu Tyr
1 5

<210> SEQ ID NO 209
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 209

Arg Ser Ser Cys Thr
1 5

<210> SEQ ID NO 210
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 210

gtagttccag ttatacc 17

<210> SEQ ID NO 211

<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 211

Val Val Pro Val Ile
1 5

<210> SEQ ID NO 212
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 212

Phe Gln Leu Tyr
1

<210> SEQ ID NO 213
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 213

Ser Ser Ser Tyr Thr
1 5

<210> SEQ ID NO 214
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 214

gtggttccgg ttatacc 17

<210> SEQ ID NO 215
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 215

Trp Phe Arg Leu Tyr
1 5

<210> SEQ ID NO 216
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 216

Gly Ser Gly Tyr Thr
1 5

<210> SEQ ID NO 217
<211> LENGTH: 17
<212> TYPE: DNA

<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 217

gtcgttccag ttatacc 17

<210> SEQ ID NO 218
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 218

Arg Ser Ser Tyr Thr
1 5

<210> SEQ ID NO 219
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 219

gtagtagctc ccactatacc 20

<210> SEQ ID NO 220
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 220

Val Val Ala Pro Thr Ile
1 5

<210> SEQ ID NO 221
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 221

Leu Pro Leu Tyr
1

<210> SEQ ID NO 222
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 222

Ser Ser Ser His Tyr Thr
1 5

<210> SEQ ID NO 223
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 223

ggcatagcag ctggtactac tacaatatcc t 31

<210> SEQ ID NO 224
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 224

Gly Ile Ala Ala Gly Thr Thr Thr Ile Ser
1 5 10

<210> SEQ ID NO 225
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 225

Gln Leu Val Leu Leu Gln Tyr Pro
1 5

<210> SEQ ID NO 226
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 226

His Ser Ser Trp Tyr Tyr Tyr Asn Ile
1 5

<210> SEQ ID NO 227
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 227

ggtatagcag ctggtactac tacaatatcc t 31

<210> SEQ ID NO 228
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 228

ggcatagcag ctggtactac tacaatatcc a 31

<210> SEQ ID NO 229
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 229

ggtatagcat acaccattag tacaatatcc t 31

<210> SEQ ID NO 230
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 230

Gly Ile Ala Tyr Thr Ile Ser Thr Ile Ser
1 5 10

<210> SEQ ID NO 231
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 231

His Thr Pro Leu Val Gln Tyr Pro
1 5

<210> SEQ ID NO 232
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 232

Tyr Ser Ile His His
1 5

<210> SEQ ID NO 233
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 233

ggtatagcat acaccaccag tacaatatct t 31

<210> SEQ ID NO 234
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 234

Gly Ile Ala Tyr Thr Thr Ser Thr Ile Ser
1 5 10

<210> SEQ ID NO 235
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 235

His Thr Pro Pro Val Gln Tyr Leu
1 5

-continued

<210> SEQ ID NO 236
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 236

Tyr Ser Ile His His Gln Tyr Asn Ile
1 5

<210> SEQ ID NO 237
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 237

ggagtagcag ctaccaccac tacaatatcc t 31

<210> SEQ ID NO 238
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 238

Gly Val Ala Ala Thr Thr Thr Thr Ile Ser
1 5 10

<210> SEQ ID NO 239
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 239

Gln Leu Pro Pro Leu Gln Tyr Pro
1 5

<210> SEQ ID NO 240
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 240

Ser Ser Ser Tyr His His Tyr Asn Ile
1 5

<210> SEQ ID NO 241
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 241

ggaatagcaa tcaccaccac aatatgct 28

<210> SEQ ID NO 242
<211> LENGTH: 9
<212> TYPE: PRT

<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 242

Gly Ile Ala Ile Thr Thr Thr Ile Cys
1 5

<210> SEQ ID NO 243
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 243

Gln Ser Pro Pro Gln Tyr Ala
1 5

<210> SEQ ID NO 244
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 244

Asn Ser Asn His His His Asn Met
1 5

<210> SEQ ID NO 245
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 245

ggaatagcag tcaccaccac aatatgct 28

<210> SEQ ID NO 246
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 246

Gly Ile Ala Val Thr Thr Thr Ile Cys
1 5

<210> SEQ ID NO 247
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 247

Gln Ser Pro Pro Gln Tyr Ala
1 5

<210> SEQ ID NO 248
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:

<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 248

Asn Ser Ser His His His Asn Met
1 5

<210> SEQ ID NO 249
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 249

ggtataataa ccactccaaa aatcgtaata c 31

<210> SEQ ID NO 250
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 250

Gly Ile Ile Thr Thr Pro Lys Ile Val Ile
1 5 10

<210> SEQ ID NO 251
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 251

Pro Leu Gln Lys Ser
1 5

<210> SEQ ID NO 252
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 252

Tyr Asn Asn His Ser Lys Asn Arg Asn
1 5

<210> SEQ ID NO 253
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 253

ggtataataa ccactccaaa aatgctaata c 31

<210> SEQ ID NO 254
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 254

-continued

Gly Ile Ile Thr Thr Pro Lys Met Leu Ile
1 5 10

<210> SEQ ID NO 255
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 255

Pro Leu Gln Lys Cys
1 5

<210> SEQ ID NO 256
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 256

Tyr Asn Asn His Ser Lys Asn Ala Asn
1 5

<210> SEQ ID NO 257
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 257

gttataataa ccagtcaaaa tatcgtaata c 31

<210> SEQ ID NO 258
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 258

Val Ile Ile Thr Ser Gln Asn Ile Val Ile
1 5 10

<210> SEQ ID NO 259
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 259

Pro Val Lys Ile Ser
1 5

<210> SEQ ID NO 260
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 260

Tyr Asn Asn Gln Ser Lys Tyr Arg Asn

-continued

1 5

<210> SEQ ID NO 261
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 261

gttataataa ctccccgaac catagtaata c 31

<210> SEQ ID NO 262
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 262

Val Ile Ile Thr Pro Arg Thr Ile Val Ile
1 5 10

<210> SEQ ID NO 263
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 263

Leu Pro Glu Pro
1

<210> SEQ ID NO 264
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 264

Tyr Asn Asn Ser Pro Asn His Ser Asn
1 5

<210> SEQ ID NO 265
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 265

gttataataa ctccccgaac atagtaatac 30

<210> SEQ ID NO 266
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 266

Val Ile Ile Thr Pro Arg Thr
1 5

-continued

<210> SEQ ID NO 267
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 267

Leu Pro Glu His Ser Asn
1 5

<210> SEQ ID NO 268
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 268

Tyr Asn Asn Ser Pro Asn Ile Val Ile
1 5

<210> SEQ ID NO 269
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 269

ggtataagca taactccccc aaacgtaatc ataatac 37

<210> SEQ ID NO 270
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 270

Gly Ile Ser Ile Thr Pro Pro Asn Val Ile Ile Ile
1 5 10

<210> SEQ ID NO 271
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 271

Leu Pro Gln Thr
1

<210> SEQ ID NO 272
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 272

Tyr Lys His Asn Ser Pro Lys Arg Asn His Asn
1 5 10

<210> SEQ ID NO 273
<211> LENGTH: 31

<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 273

gtagtaataa ccactactat catagtaata c 31

<210> SEQ ID NO 274
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 274

Val Val Ile Thr Thr Thr Ile Ile Val Ile
1 5 10

<210> SEQ ID NO 275
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 275

Pro Leu Leu Ser
1

<210> SEQ ID NO 276
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 276

Ser Asn Asn His Tyr Tyr His Ser Asn
1 5

<210> SEQ ID NO 277
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 277

gtagttactg tagtca 16

<210> SEQ ID NO 278
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 278

Val Val Thr Val Val
1 5

<210> SEQ ID NO 279
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:

<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 279

Ser Tyr Cys Ser
1

<210> SEQ ID NO 280
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 280

gtagtcaccg tagtca 16

<210> SEQ ID NO 281
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 281

Ser His Arg Ser
1

<210> SEQ ID NO 282
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 282

ggagttacca ccgtagtca 19

<210> SEQ ID NO 283
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 283

Gly Val Thr Thr Val Val
1 5

<210> SEQ ID NO 284
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 284

Glu Leu Pro Pro
1

<210> SEQ ID NO 285
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 285

Ser Tyr His Arg Ser
1 5

<210> SEQ ID NO 286
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 286

gtaaccatag ctgtatccac 20

<210> SEQ ID NO 287
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 287

Val Thr Ile Ala Val Ser
1 5

<210> SEQ ID NO 288
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 288

Asn His Ser Cys Ile His
1 5

<210> SEQ ID NO 289
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 289

gtaatcgtag ccactatatc cac 23

<210> SEQ ID NO 290
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 290

Val Ile Val Ala Thr Ile Ser
1 5

<210> SEQ ID NO 291
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 291

Pro Leu Tyr Pro
1

<210> SEQ ID NO 292
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 292

Asn Arg Ser His Tyr Ile His
1 5

<210> SEQ ID NO 293
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 293

gtaattgtag ccatctctac 20

<210> SEQ ID NO 294
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 294

Val Ile Val Ala Ile Ser
1 5

<210> SEQ ID NO 295
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 295

Asn Cys Ser His Leu Tyr
1 5

<210> SEQ ID NO 296
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 296

ggacgagctg ctatactc 18

<210> SEQ ID NO 297
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 297

Gly Arg Ala Ala Ile Leu
1 5

<210> SEQ ID NO 298

-continued

<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 298

Asp Glu Leu Leu Tyr
1 5

<210> SEQ ID NO 299
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 299

Thr Ser Cys Tyr Thr
1 5

<210> SEQ ID NO 300
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 300

gtaccagctg ctgctatacc c 21

<210> SEQ ID NO 301
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 301

Val Pro Ala Ala Ala Ile Pro
1 5

<210> SEQ ID NO 302
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 302

Tyr Gln Leu Leu Leu Tyr
1 5

<210> SEQ ID NO 303
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 303

Thr Ser Cys Cys Tyr Thr
1 5

<210> SEQ ID NO 304
<211> LENGTH: 21
<212> TYPE: DNA

<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 304

gtaccagcca ctgctatacc c 21

<210> SEQ ID NO 305
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal'

<400> SEQUENCE: 305

Val Pro Ala Thr Ala Ile Pro
1 5

<210> SEQ ID NO 306
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 306

Tyr Gln Pro Leu Leu Tyr
1 5

<210> SEQ ID NO 307
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 307

Thr Ser His Cys Tyr Thr
1 5

<210> SEQ ID NO 308
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 308

gtagccgctg ctataccc 18

<210> SEQ ID NO 309
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 309

Val Ala Ala Ala Ile Pro
1 5

<210> SEQ ID NO 310
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

-continued

<400> SEQUENCE: 310

Pro Leu Leu Tyr
1

<210> SEQ ID NO 311
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 311

Ser Arg Cys Tyr Thr
1 5

<210> SEQ ID NO 312
<211> LENGTH: 366
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 312

caggtgcagc tacagcagtg gggcgcagga ctgttgaagc cttcggatac cctgtccctc 60

acctgcgctg tctatggtgg gtccttcagt ggttactact ggagctggat ccgccagccc 120

ccagggaagg ggctggagtg gattggggaa atcaatcata gtggaagcac caactacaac 180

ccgtccctca agagtcgagt caccatatca gtagacacgt ccaagaacca gttctccctg 240

aagctgagct ctgtgaccgc cgcggacacg gctgtgtatt actgtgcggg gcatagccat 300

ggctggtact actactacta cggtatggac gtctggggcc aagggaccac ggtcaccgtc 360

tcctca 366

<210> SEQ ID NO 313
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 313

Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Asp
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Gly His Ser His Gly Trp Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val Trp
100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 314
<211> LENGTH: 96
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 314

Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

<210> SEQ ID NO 315
<211> LENGTH: 355
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 315

caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcacagac cctgtccctc 60

acctgcactg tctctggtgg ctccatcagc agtggtggtt actactggag ctggatccgc 120

cagcacccag ggaagggcct ggagtggatt gggtacatct attacagtgg gagcacctac 180

tacaacccgt ccctcaagag tcgagttacc atatcagtag acacgtctaa gaaccagttc 240

tccctgaagc tgagctctgt gactgccgcg gacacggccg tgtattactg tgcgaggggg 300

gaccacggtc actacgacta ctggggccag ggaaccctgg tcaccgtctc ctcag 355

<210> SEQ ID NO 316
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 316

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
20 25 30

Gly Tyr Tyr Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu
35 40 45

Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Gly Asp His Gly His Tyr Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> SEQ ID NO 317
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 317

-continued

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
            20                  25                  30

Gly Tyr Tyr Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu
        35                  40                  45

Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65                  70                  75                  80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95

Cys Ala
```

<210> SEQ ID NO 318
<211> LENGTH: 370
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 318

```
cagctgcagc tgcaggagtc gggcccagga ctggtgaagc cttcggagac cctgtccctc     60

acctgcactg tctctggtgg ctccatcagc agtagtagtt actactgggg ctggatccgc    120

cagcccccag ggaaggggct ggagtggatt gggagtatct attatagtgg gagcacctac    180

tacaacccgt ccctcaagag tcgagtcacc atatccgtag acacgtccaa gaaccagttc    240

tccctgaagc tgagctctgt gaccgccgca gacacggctg tgtattactg tgcgagacat    300

gaagggcata gccaccttaa ctggttcgac ccctggggcc agggggggaac cctggtcacc    360

gtctcctcag                                                           370
```

<210> SEQ ID NO 319
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 319

```
Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser
            20                  25                  30

Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35                  40                  45

Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65                  70                  75                  80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95

Cys Ala Arg His Glu Gly His Ser His Leu Asn Trp Phe Asp Pro Trp
            100                 105                 110

Gly Gln Gly Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> SEQ ID NO 320
<211> LENGTH: 99
<212> TYPE: PRT

-continued

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 320

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser
20 25 30

Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45

Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg

<210> SEQ ID NO 321
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 321

tcttatcaga caggggctc tc 22

<210> SEQ ID NO 322
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal

<400> SEQUENCE: 322

ggaagacatt tgggaaggac tg 22

<210> SEQ ID NO 323
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Mammal
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: wherin the "Xaa" represents any amino acid

<400> SEQUENCE: 323

Phe Gly Xaa Gly
1

<210> SEQ ID NO 324
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Mammal
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: wherein the "Xaa" represents any amino acid

<400> SEQUENCE: 324

Trp Gly Xaa Gly
1

What is claimed is:

1. A non-human animal comprising

(i) in its germline genome a genetically modified immunoglobulin heavy chain locus comprising an unrearranged human immunoglobulin heavy chain variable region nucleotide sequence, wherein the unrearranged heavy chain variable region nucleotide sequence comprises an addition of at least one histidine codon or a substitution of at least one non-histidine codon with a histidine codon, wherein the histidine codon is not encoded by a corresponding human germline heavy chain variable region gene segment; and

wherein the added or substituted histidine codon is present in a complementary determining region 3 (CDR3) encoding sequence.

2. The non-human animal of claim **1**, wherein the non-human animal is a mammal.

3. The non-human animal of claim **2**, wherein the mammal is a rodent selected from the group consisting of a mouse, a rat, and a hamster.

4. The non-human animal of claim **1**, wherein the CDR3 encoding sequence is selected from a human V_H gene segment sequence, a human D gene segment sequence, a human J_H gene segment sequence, and a combination thereof.

5. The non-human animal of claim **1**, wherein the CDR3 encoding sequence is selected from a human germline V_H gene segment sequence, a human germline D gene segment sequence, a human germline J_H gene segment sequence, and a combination thereof.

6. The non-human animal of claim **5**, further comprising at least a second additional or substituted histidine codon in at least one reading frame of the human immunoglobulin heavy chain gene segment that encodes a heavy chain variable domain selected from an N-terminal region, a loop 4 region, a complementary determining region 1 (CDR1), a complementary determining region 2 (CDR2), the complementary determining region 3 (CDR3), and a combination thereof.

7. The non-human animal of claim **1**, wherein the endogenous non-histidine codon that is substituted with the histidine codon encodes the amino acid selected from the group consisting of Y, N, D, Q, S, W, and R.

8. The non-human animal of claim **1**, wherein the added or substituted histidine codon is present in at least one reading frame of a human D gene segment.

9. The non-human animal of claim **8**, wherein the reading frame is a hydrophilic frame of the human D gene segment, and the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, and a combination thereof.

10. The non-human animal of claim **1**, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence.

11. The non-human animal of claim **10**, wherein the unrearranged human immunoglobulin heavy chain variable region nucleotide sequence is operably linked to an endogenous non-human heavy chain constant region nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

12. The non-human animal of claim **10**, wherein the human heavy chain constant region nucleotide sequence comprises a modification that increases an affinity of a C_H2-C_H3 region of an IgG heavy chain constant region amino acid sequence to neonatal Fc receptor (FcRn) at a pH ranging from about 5.5 to about 6.0, wherein the modification is a mutation in the IgG heavy chain constant region amino acid sequence selected from the group consisting of M428L, N434S, V259I, V308F, N434A, M252Y, S254T, T256E, T250Q, H433K, N434Y, and a combination thereof.

13. The non-human animal of claim **1**, wherein the non-human animal is homozygous for the genetically modified immunoglobulin heavy chain locus in the germline.

14. The non-human animal of claim **1**, wherein the non-human animal further comprises an unrearranged human immunoglobulin light chain V gene segment and an unrearranged human immunoglobulin light chain J gene segment.

15. The non-human animal of claim **1**, wherein the non-human animal comprises a B cell population that is capable of producing a diverse population of antigen-binding proteins that exhibit pH-dependent binding, each comprising a heavy chain variable domain having at least one histidine residue derived from the added or substituted histidine codon.

16. The non-human animal of claim **15**, wherein at least one B cell of the B cell population comprises a rearranged human immunoglobulin heavy chain variable region sequence that is derived from the modified immunoglobulin heavy chain locus and that comprises at least one somatic hypermutation (SHM).

17. The non-human animal of claim **15**, wherein the antigen-binding protein produced by the B cell population exhibits a decreased antigen-binding affinity at a pH ranging from about 5.5 to about 6.0 as compared with at a neutral pH ranging from about 7.0 to about 7.4.

18. The non-human animal of claim **1**, wherein the non-human animal is heterozygous for the genetically modified immunoglobulin heavy chain locus in the germline.

19. The non-human animal of claim **1**, wherein the non-human animal comprises an Adam6a gene, an Adam6b gene, or both.

20. A method of making a non-human animal that comprises a genetically modified immunoglobulin heavy chain locus in its germline genome, the method comprising:

(a) modifying a genome of a non-human animal to delete or render non-functional endogenous immunoglobulin heavy chain V, D, and I gene segments in an immunoglobulin heavy chain locus; and

(b) placing in the genome an unrearranged human heavy chain variable region nucleotide sequence comprising an addition of at least one histidine codon or a substitution of at least one endogenous non-histidine codon with a histidine codon, wherein the histidine codon is not encoded by a corresponding human germline heavy chain variable region gene segment; and wherein the added or substituted histidine codon is present in a complementary determining region 3 (CDR3) encoding sequence.

21. The method of claim **20**, wherein the CDR3 encoding sequence is selected from a human V_H gene segment, a human D gene segment, a human J_H gene segment, and a combination thereof.

22. The method of claim **20**, wherein the CDR3 encoding sequence is selected from a human germline V_H gene segment sequence, a human germline D gene segment sequence, a human germline J_H gene segment sequence, and a combination thereof.

23. The method of claim **20**, further comprising at least a second additional or substituted histidine codon in at least one reading frame encoding a heavy chain variable domain selected from an N-terminal region, a loop 4 region, a complementary determining region 1 (CDR1), a complementary determining region 2 (CDR2), the complementary determining region 3 (CDR3), and a combination thereof.

24. The method of claim **20**, wherein the endogenous non-histidine codon that is replaced with the histidine codon encodes the amino acid selected from the group consisting of Y, N, D, Q, S, W, and R.

25. The method of claim **20**, wherein the added or substituted histidine codon is present in one or more reading frame of a human D gene segment.

26. The method of claim **25**, wherein the reading frame is a hydrophilic frame of the human D gene segment, and the hydrophilic frame comprises a nucleotide sequence that encodes the amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, and a combination thereof.

27. The method of claim **20**, wherein the unrearranged immunoglobulin human heavy chain variable region nucleotide sequence is operably linked to a human or non-human heavy chain constant region nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

28. The method of claim **27**, wherein the unrearranged immunoglobulin human heavy chain variable region nucleotide sequence is operably linked to an endogenous non-human heavy chain constant region nucleotide sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

29. The method of claim **27**, wherein the human heavy chain constant region nucleotide sequence comprises a modification that increases an affinity of a C_H2-C_H3 region of an IgG heavy chain constant region amino acid sequence to neonatal Fc receptor (FcRn) at a pH ranging from about 5.5 to about 6.0, wherein the modification is a mutation in the IgG heavy chain constant region amino acid sequence selected from the group consisting of M428L, N4345, V2591, V308F, N434A, M252Y, 5254T, T256E T250Q, H433K, N434Y, and a combination thereof.

30. The method of claim **20**, wherein the non-human animal is homozygous for the genetically modified immunoglobulin heavy chain locus in the germline genome.

31. The method of claim **20**, wherein the non-human animal comprising the genetically modified immunoglobulin heavy chain locus comprises a B cell population that is capable of producing an diverse population of antigen-binding proteins that exhibit pH-dependent binding, each comprising a heavy chain variable domain having at least one histidine residue derived from the added or substituted histidine codon.

32. The method of claim **31**, wherein at least one B cell of the B cell population comprises a rearranged human immunoglobulin heavy chain variable region sequence that is derived from the modified immunoglobulin heavy chain locus and that comprises at least one somatic hypermutation (SHM).

33. The method of claim **31**, wherein the antigen-binding protein produced by the B cell population exhibits a decreased antigen-binding affinity at a pH ranging from about 5.5 to about 6.0 as compared with at a neutral pH ranging from about 7.0 to about 7.4.

34. The method of claim **20**, wherein the non-human animal comprises an Adam6a gene, an Adam6b gene, or both.

35. The method of claim **20**, wherein the method results in a genetically modified animal that comprises a population of B cells for antibodies exhibiting enhanced pH-dependent binding to an antigen of interest.

* * * * *